CANCER RESEARCH LABORATORIES

OF THE

BARNARD FREE SKIN AND CANCER HOSPITAL

AND

THE DEPARTMENT OF SURGERY

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

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VOLUME II

To the Board of Directors and Medical Board of the Barnard Free Skin and Cancer Hospital, and the Department of Surgery, Washington University School of Medicine, Saint Louis, Missouri.

GENTLEMEN:—

The work has progressed steadily since the publication of our last volume of reprints. In this volume we include reprints of articles appearing since December, 1923. This later work has confirmed our earlier deduction that cancer is the result of a crowding of cells together and a relative reduction in the circulation to the mass thus formed. It is not the result of a primary change in the cells, but of an overcrowding of cells and stagnation. These factors are important because growth depends on the accumulation and concentration of a certain substance formed as cells oxidize food material. This substance has been called the archusia (S). It is removed from the cells by the active blood circulation of the normal organism.

In analyzing for the nature of the archusia, we have found that it is formed by all cells in nature and is the vitamin B of food. Vitamin A is, on the other hand, a product of the growth reaction of cells. Cancer is the result of an excessive local increase in vitamin B. Bacteria and animal parasites lead to the production of cancer in that they liberate an excess of vitamin B in their growth. X-rays and radium* induce the formation of vitamin B in the tissues. Coal tar induces cancer in that it dissolves and removes the vitamin A from the tissues about it. Cancer may be thus reduced to terms of a local imbalance of the vitamin content of the tissues.

These studies reduce cancer, therefore, for the first time to terms which can be readily correlated with the normal conditions of the life of man and other diseases peculiar to him. In them lies also the immediate hope for the development of means for the prevention and cure of this disease.

*The experiments showing the action of the x-rays in the liberation of vitamin B in the tissues have been reported by Dr. Edwin C. Ernst before the Int. Cong. of Radiology in London, July, 1925, and before the Radiological Society of North America, December 12, 1925. The reprints of these studies will be included in the next volume.

It is with pleasure that we submit this report to you. It has been your encouragement and generous support which has made this work possible.

Respectfully submitted,

Director of the Research Laboratories of the Barnard Free Skin and Cancer Hospital, and Assoc. Prof. of Experimental Surgery, Washington University School of Medicine, Saint Louis, Missouri.

Saint Louis, Missouri,
December 31, 1925.

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FACTORS REGULATING CELLULAR GROWTH AND THEIR IMPORTANCE IN THE EXPLANATION OF CANCER*

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As has been pointed out in a previous article,[1] not only the earlier work on cancer but also the recent careful studies of this disease have indicated that its cure and control will not be solved alone through the identification of the conditions which lead to its production. Its solution will be the natural outcome of the eventual determination of the structure and metabolism of body cells. Cancer, unlike inflammatory processes, is not a reaction itself to certain irritating substances, but it is the result of a change either in the cell or tissue which when once established continues independent of the original causative agent or agents. It is an autonomous growth of certain body cells which continues like the growth of parasites to the destruction of neighboring tissues and organs and takes place at the expense of the nutrition of the whole.

This deduction is clearly indicated by earlier studies and more definitely proven by many recent investigations. These later clinical and experimental studies have shown that cancer may be induced by a variety of substances and conditions, such as coal tar, paraffin, other lipoid solvents, arsenic, certain animal parasites, the roentgen ray, radium, etc.; and that it may arise spontaneously in the course of the life of individuals of certain families of mice, and in certain congenital tumors and defects and in old inflammatory processes in man. If coal tar be painted on the skin of a rat repeatedly over a long enough time, cancer invariably results. This cancer once established, unlike inflammatory processes, then proceeds independent of the tar. Pieces of the tissue may be transplanted to other animals and so the disease may be propagated through many generations of animals. It is too late for the pipe smoker to discard his pipe after the cancer has developed. An irretrievable change has already been established.

The problem in cancer as it appears today is the same as it presented itself to many of the earlier authors. It is the problem of the general conditions which regulate cellular growth in the organism.

As is well known, an active independent growth of cells in the body is peculiar alone, under normal conditions, to the earlier periods of development. At first this growth is quite generalized. Later it becomes localized, first in one part and then in another. In man it ceases entirely with the laying down of the last kidney tubule and glomerulus at about ten days after birth. Subsequent to this time all growth is merely the enlargement of preformed organs and tissues. It is like the hypertrophies and hyperplasias of later life.

In later life it is again well known that hypertrophy and hyperplasia are related directly to the functional activity of the part. Increase the work of the heart, it grows. Decrease its activity, it atrophies. So, in the same manner, the removal of a part of any organ or another organ of the same kind leads to an active enlargement of the remaining parts. This enlargement continues to the re-establishment of a certain size which corresponds to the functional demand made upon the part.

As a moderate increase in the functional demands made upon an organ or a part is associated with the growth of that organ or part so further or excessive work is associated with a degeneration of these same cells. Such a degeneration is also seen in certain structures during development. The pronephros and the metanephros of higher animals suffer such changes as they give way for the development of the kidney.

Cancer represents a return of the property of independent active growth to certain cells, or rather to groups of certain cells in the organism. This independently growing tissue also suffers extensive degenerative changes in part. While the degenerations resemble ordinary autolytic changes, they differ from those seen in the infarct, for instance, in that they are not harmful for the organism. The disappearance of the pronephros or the metanephros does not disturb the nutrition of the whole. So it has been shown that large tumors may disappear in a few days without evident toxic symptoms to the animal.[2]

Closely associated with this loss of independent active growth in the developing organism marked morphological and chemical

*Read in Section on Pathology, Southern Medical Association, Seventeenth Annual Meeting, Washington, D. C., Nov. 13-15, 1923.

also take place in the tissue. The various cells peculiar to the different organs and tissues are differentiated. The blood vascular system develops. The cells become separated by a rich vascular network and by a deposit of extracellular reticular fibres, the connective tissue fibrils. In early embryonic life the cells are closely packed together. The number of capillaries per unit cell area is small. In later life one sees great variation in the number of cells per unit area in any tissue. In the most cellular tissue of this period all cells are closely beset with capillaries. The cells of the liver, for instance, are separated into columns no more than two cells thick. The circulation is active in these capillaries. This is in contrast to the slow, sinusoidal circulation of the embryonic liver.

Such an arrangement of cell peculiar to the adult is strikingly different, therefore, from the dense cell masses of cancer and early life. Cancerous tissue is peculiar not only in that it grows actively and independently of the whole or of any functional demand made upon its cells, but it again, like the tissues of early embryonic life, is a densely cellular tissue. It is peculiar not that its cells are like the embryonal cells—they are not—but that they are arranged in dense masses. They have lost in many instances the shape peculiar to them in the normal tissues. Otherwise they are not embryonal. The cells of the cancer of the skin are skin epithelial cells. So the cells of the sarcoma are connective tissue cells. The only demonstrable change they have suffered is in their arrangement, their ability to grow and their shape.

Cohnheim, like many older authors and a large modern school, has taken the view that this loss of independent growth of cells in early embryonic life is the direct result of some primary age-change in the cell. They have ignored largely the accompanying changes in the environment, the gradual separation of the cells by intercellular substance and blood vessels. They have considered that the loss of independent growth during development is the direct result of a differential change the cells suffer or that it is due to an aging of the cell. Cohnheim, therefore, introduced the view that cancers must always arise from embryonal cells which have lain latent in the tissue and have not suffered such age-changes. The failure to have found any proof of the existence of such cells has led other authors to believe that this active independent growth in cancer is due to a special differentiation in the cells, a hyperdifferentiation, for instance, in the sense of Robertson,[3] or otherwise. The coal tar and other specific substances act, therefore, only to change the general structure of the cell, to remove special inhibitors, or to bring about a special differentiation in these cells.

All attempts to prove these later ideas have also failed. Hanselman thought he had proven such a differentiation in the discovery of abnormal mitoses in cancer cells. Further careful studies have fully shown, however, that such abnormal mitoses are readily induced in any normal cells by merely changing the concentration of many stimulants capable of inducing normal divisions in these cells. Other studies of body cells have fully shown their fluid nature. This indicates clearly that their shape at all times is dependent upon their immediate environment. The difference in the shape of the normal cell from that of the cancer cell may be ascribed to a change in the general organization of the tissues rather than to any primary change in the cell. So in the same manner all other changes such as the change in the nuclear-cytoplasmic relationship have not been proven to be important. In fact, the better work of the last and this Century has shown quite clearly, as E. B. Wilson points out, that growth, differentiation and function are not determined by the cell, but by more general forces or stimuli active in the organism. Driesch showed when the first two blastomeres of the eggs of sea urchins are separated each will develop into a mature individual. He states:

"The relative position of a blastomere in the whole determines in general what develops from it; if its position be changed it gives rise to something different."[4]

As Wilson has clearly pointed out, cell-division in the organism at all times is determined by the environment and not the cell. He states that all evidence points to the fact that

"cell-formation is subordinate to growth, or rather to the general formative process of which growth is an expression."[5]

What these earlier authors failed to find is the nature of the conditions leading to each of these various changes that the cells may suffer. Driesch noted that the normal growth of an animal is related to a special form. He found that, when the gastrula of the sea urchin is bisected, each half does not grow at once, but only after each has reformed by a shifting of its cells into a perfect gastrula of one half the size. These observations of Driesch have been fully confirmed by Miss Bickford and Morgan for the regeneration of the hydranths of tubularians and for the regeneration of planaria from pieces of these animals. No growth takes place in the pieces until a perfect hydranth or animal of small size is reformed by a shifting of the cells.[4]

What Driesch failed to find were the conditions which led to the primary remolding. He looked at the remolding as the work of some vital force. That growth was dependent upon this primary remolding, or a special arrangement of cells, seemed certain to him.

The tissue culture has not only given more definite proof for the relation of the general

organization of the animal or part to function on the one hand and growth on the other, but it has allowed us to analyze directly the nature of the environmental changes suitable for each form of activity. As I pointed out in 1913, and later in 1915, rhythmical muscular contraction develops only in a cell of the tissue culture which becomes stretched through a serum cavity between the tissue fragment or the surface of the medium and a band of elastic fibrin attached to its other end. If this same cell be simply transferred from this position to the side of the band of fibrin it takes on all the properties of a simple connective tissue cell. If it be laid near a cellular fragment about which the cells are growing it grows and divides actively and has all the appearance and activities of a sarcoma cell.[5] [6] [7] [8]

So in the same manner, as I have pointed out in a recent paper, all functional activity in the organism can be related to an orderly laying down of extracellular materials, the connective fibrils, the basement membranes, the nerve and muscle sheaths, etc.[9]

That the shape of the cells and their general arrangement in the normal organism and cancer is to be related directly to the environment I was able also to show in the earlier work with the tissue culture. All cells suspended in a liquid round off to perfect spheres. These cells take shape only when brought into contact with surfaces and the shape assumed is directly dependent upon the shape of the surface of contact and other demonstrable forces in the culture.[10] This applies not only to the connective tissue cells, but to all types of cells. The connective tissue cells take a spindle shape in a culture medium of blood plasma, in that they are able to coagulate the plasma, form fibrin fibrils and stick to the side of these fibrils. Lymphoid and other wandering cells have no such property of fibrin formation. They cannot stick to fibrin formed by the connective tissue cells. They remain spherical or ovoid in shape when placed in the plasmatic medium. In the same manner the shape of the epithelial cells may be reduced to physio-chemical expression.[11]

In a medium of blood plasma, an environment strange for these glandular tissues, the epithelial cells rarely ever grow out as tubes. Carrel and I noted tubules to form about one fragment of thyroid, but in most cases the cells spread out as broad thin sheaths of cells like the epithelium of the skin. Champy, in 1919,[12] showed that if connective tissue cells be added to a culture of kidney the tubular formation will be regained in the epithelial cells of the culture. Drew, in 1923,[13] confirmed these observations of Champy.

All of these facts stand as direct proof of my earlier observations and indicate clearly that growth, function and cellular shape are not changes related to the age of the cell, but its immediate environment. In 1915, I[14] again gave evidence to show that rhythmical contractions are not the work of any cellular machine, but that it is the response of the cell to a particular environment. In the same manner it seemed evident that an active independent growth may be merely another form of reaction that the cell may undergo in another environment.

As I noted above, the disappearance of the active independent growth of cells in the developing organism may be directly related to the separation of the cells by a rich vascular network and intercellular materials. Further studies of the process of rhythmical contraction in heart muscle cells have shown that this functional state is dependent not only upon extracellular materials arranged in a certain relation to the cell, but also upon an active circulation. In simple hanging drop cultures, where there is no means of interchange about the cells except by diffusion, the contractions of muscle cells become irregular. There are periods of activity followed by periods of rest. The active periods are ushered in by rapid strong contractions. These become weaker and weaker until they finally cease for a time. After a short or longer rest period they begin again.

If a stream of serum be passed over these same cells these irregularities cease. The cells contract forcibly and continuously under such conditions until their supply of nutrition is exhausted. Stop this flow of serum and these irregularities appear immediately again.

Quite the reverse to this peculiarity of the functional states in the organism I have found, and noted in a recent paper,[15] that growth is inhibited by such a stream of serum passing over the culture.

For many years it has been known that increased functional activity in the organism is always associated with an increased circulation to the part. Growth, however, may follow a decrease in the circulation. The clubbing of the fingers with disturbances in the general circulation illustrates clearly this fact. In an unpublished paper on the circulation of the nail I noted that the circulation of the growing bed is very much less than along the whole of the outer parts of this structure.[16] The entire growth of the developing nervous system takes place along the central canal. The blood vessels invade the outer peripheral portions. In the light of these facts it may not be surprising, therefore, that the heart hypertrophies when it is overloaded, when its cavities are dilated and its walls compressed. Such compression of the walls must impede the circulation of the muscle fibres.

In the tissue culture I pointed out as early as 1913[17] [18] that the rate of growth activity of any

particular tissue is directly proportional to the size of the fragments, provided fragments too large for oxygen to diffuse to all of their parts are not used. Single cells cannot grow in the cultures. Growth takes place always about fragments of tissues.

Again it has been noted that this growth and the whole metabolic activity of the cell is greatest about fragments of young embryonic tissue and fragments of cancer. In the cases of embryonic tissue this ability to grow decreases with age. It is less in extent about fragments of tissues of older embryos than about similar fragments of younger ones planted in the same medium. The cells from the older tissues not only show a decrease in ability to grow in the same medium, but this activity does not commence so early in the cultures about the older tissue fragments as about the younger ones. In direct proportion to the age and the decrease in activity, there is a corresponding increase in the latent period before any activity commences after the cultures are prepared.

At first I thought that these changes in relation to growth were to be related directly to an aging of the cells. It soon became evident that this was not true. It was not in any way related to the cell, but to the density of the cells in the fragments. If the cells in these younger embryonic fragments be carefully teased apart, and then washed with salt solution, their reaction becomes identical with the cells of older fragments in which the cells are normally separated in the same manner by intercellular material.

A further careful study of fragments of tuberculous granulation tissue has shown that the reaction of the connective tissue of the fragments in cultures is identical with the embryonic and adult cells of fragments having an equal cell content. Tuberculous granulation tissue, as is well known, varies in cellular content in different individuals and in different parts of the same mass of tissue. The cells react about the cellular fragments like those from equally cellular fragments of tissue of the embryos. About the more fibrous fragments this reaction is like the reaction of the cells from fragments of subcutaneous tissue in which the cells are equally separated by intercellular fibrils[1] and blood vessels. In the same manner the growth is more active about fragments of a cellular sarcoma than about the fragments of a fibrosarcoma, and this growth of the cancerous tissue, like that of normal tissue, is in every case proportional to the number of cells per unit area in the fragment and similar to a normal tissue having an equal cell content and vascular supply. The isolated cancer cell washed with serum or other isotonic solution reacts like any isolated normal cell. The difference between the cancerous tissue and the normal adult tissue is that the former is charged with products of the cell's metabolism, which have not been able to escape. The embryonic tissue is like the cancerous tissue except that it contains a greater amount of nutrient substances, the yolk. The embryonic cells will survive longer than the cancer cells in a medium of blood plasma which contains little or no nutrient substance.

In 1911, Carrel and I[19] had already shown that extracts of embryonic tissue and cancer contain an active growth stimulant. Drew,[13] at the Imperial Cancer Research Laboratory, has recently repeated and confirmed these experiments. When extracts of such tissue made in simple isotonic sodium chlorid solution are added to the medium of another culture the growth is greatly accelerated. It is interesting to note here that these same accelerating substances will accumulate in any fragment of older tissue if the fragments are cut off for a time from their blood supply and placed in the culture. This is well illustrated by the latent period noted in cultures of older tissue as well as in studies of extracts of any of these tissues. A study of the effect on other cells of extracts in salt solution of fragments of any normal adult tissue which has been removed from the body and placed for a time in a culture has shown that these fragments as they lay in the stagnant culture become slowly charged with these extractable stimuli or this stimulus.

While Carrel's and my earlier experiments had clearly indicated that the growth in cancer may be the direct result of a stimulus, they led us nowhere, because they did not indicate the source of this stimulus. Thiersch and Beneke many years ago thought that tumor growth may be a degenerative overgrowth of cells. Oertel took a similar view.[2] Recently Drew claims that autolysing tissues contain a stimulus.

In my first paper on tissue culture, in 1911,[11] I showed that autolysis as it results from the absence of oxygen is in no way concerned with the growth process. Cellular growth is strikingly inhibited about fragments of nervous tissue so large that oxygen cannot diffuse readily to all of their parts. As I later showed, in 1913,[17] growth[18] is maximum only about the largest fragment into which oxygen may diffuse readily to all its parts.

Carrel's and my experiments showed that the substances which accelerate growth are soluble in simple salt solution. I find them also soluble in serum and plasma, or in the circulating blood of the body. As I noted above, growth is accelerated by cutting down the circulation. In tissue cultures the growth is greatest about the fragments which are placed in simple hanging drop cultures and are not disturbed. Simple shaking of the serum which accumulates on the lower surface of a hanging drop of plasma will disturb growth. It lessens it for a time.

Again, as I pointed out above, growth in the adult organism follows increased functional activity of a part. Such an increased functional ac-

tivity is associated with increased metabolism or energy production in the parts. The energy for such functional activity in the body is derived from chemical reaction. Any increase in function means an increase in the products formed in reactions and a temporary increase of them in the tissues, even in the presence of an active blood supply. This active blood supply must soon relieve the tissue of these substances. Any decrease in the circulation, on the other hand, must lead to the accumulation of these products, even when the metabolic activity is low. In cancerous tissues and in tissues of young embryos these stimulating substances are always present. The tissues are characterized by a poor circulation in relation to the number of cells per unit area. They are characterized by dense masses of cells separating the blood capillaries. The cells in the older tissues of the normal organism are separated by a rich vascular network and intercellular materials. When fragments of cancerous tissues and tissues from young embryos are removed from the body and their circulation is destroyed they become active at once in the culture or within one or two hours after the cultures are prepared. Other tissues show no activity until after a long latent period. The length of this period is directly proportional to the original cell content and vascular supply of the tissues. Before the cells can grow the proper stimulus must accumulate. In my earlier studies, in 1913,[17] I[18] showed that oxygen is necessary for the growth of cells and further, as was noted above, that autolysis from lack of oxygen is decidedly harmful. In 1917 I[20] showed, however, that the amount of oxygen necessary for tissue activity is not great. Two years ago I[21] again gave evidence to show that the accumulation of the growth stimulus is absolutely dependent upon the presence of oxygen while the growth reaction itself is not dependent upon this gas. Fragments of young embryonic tissue will show a limited growth in the absence of oxygen. Older tissues demand oxygen in the same proportion as their latent period for growth increases in the presence of a normal oxygen supply. Drew's contentions of an autolytic source for these stimulants is entirely unfounded. His own experiments disprove this deduction. He extracted the stimulant from a kidney left for one hour in the incubator. This is not long enough for autolysis to set in.

The active stimulus for growth noted in cancerous and young embryonic tissues is the normal soluble product of the metabolism of the cells. These tissues grow because their cells are crowded together and removed from an active blood supply. They are free from the intercellular substances upon which the shape, the arrangement and the functional activity of the cell depends.

In the animal organism life does not manifest itself the same from the beginning to the end. In the beginning, it is represented by an active independent growth of undifferentiated cells. Only with the changing organization of the whole do differentiation and function make their appearance. The presence of an active independent growth of cells in early life is directly dependent upon the densely cellular character of the tissue and the limited blood supply. Its disappearance in later life is the result of the development of intercellular substances and a richer blood supply. Life maintains in this later period solely from the fact that these cells become polarized and assume other forms of work consistent with these changes.

As far as the growth of body cells is concerned, it does not differ qualitatively, therefore, in any way from that of the unicellular organism. As early as 1902, Wildiers[22] noted that when a few yeast cells are introduced into a large amount of medium containing all the necessary nutrient substances they will not grow. For growth to take place there must be a certain bulk relationship between the number of cells and the medium. Wildiers and others have shown that this is due to a decrease in the concentration of some substances liberated by the yeast into the medium. A few cells may be made to grow in a large hulk of medium if an extract of yeast is added to it.

I have found that single cells of the body may be made to grow in the medium of a tissue culture if extracts of actively growing tissues are added to the medium. Embryonic extracts not only stimulate but prolong the growth. Cancerous extracts stimulate growth, but do not prolong it. They contain little or no nutrient substances.

Robertson[23] again recently, in 1921, noted that the same relationship exists in the case of the paramecium. The rate of growth at all times is directly proportional to the number of individuals per unit volume of medium until the medium becomes charged with waste products above a certain concentration. The same is known also to be true for bacteria. Webb, Williams and Barber,[24] in 1909, noted that a single tubercle bacillus or an anthrax spore introduced into a guinea pig or a mouse will not grow. Several must be injected to cause infection.

Devloo,[25] in analyzing the active substance noted by Wildiers in the cultures of yeast, came to the conclusion that it is either a phospholipin or is associated with or dissolved in lecithin. In my studies of these cells of higher animals I identified a substance liberated by body cells which is an active blood coagulant and otherwise has the physical properties of a phospholipin. This substance I have been able to

show produces quantitatively the energy for migration and muscular contraction and combines to form the extracellular fibrils of connective tissue. In cellular growth it is formed in excess and disappears, so it may likewise combine in the reaction forming protoplasm or parts of the protoplasm of the cells. This is not the stimulating substance. The stimulating substance is soluble in salt solution. It acts, in low concentration, for the formation of the energy producing substances. In higher concentration, it acts to break down the proteins in the cell and destroy the cell. It is not a food. It is a part of the cell's mechanism for migration, a part of the cell's mechanism by which it digests its food and expands or grows, and it is the substance which destroys the cells when they become too crowded in a medium supplied with oxygen. It is not surprising, therefore, that in the more crowded areas of cancerous tissue the cells degenerate, and, in the less crowded areas, they grow actively. Again it is not surprising that this degeneration in the cancerous tissue does not liberate the same products as the autolytic type resulting from the absence of oxygen. In the normal body ample nutrient substances for such a growth are always present.

Again, there is no evidence that this stimulating substance combines in any of the reactions of the cell. It acts as a catalyzer of other reactions of the cell. Its action is wholly dependent on its concentration. Its concentration at all times is directly proportional to the rate of its production and indirectly proportional to the avenue for its escape by way of the blood stream. This substance is the normal product of that part of cell metabolism into which oxygen enters. The disappearance of an independent growth in the embryo is not due to any differential change in the cell. The differentiated cells of the organism may grow as well as those of early life. The disappearance of an independent growth in normal development is to be related to the development of intercellular materials and a rich blood supply. These structures lead to the rapid removal of the necessary stimulating substance as it is formed at all times in the tissues.

Cancer, therefore, may be nothing more than the result of any condition leading to a breakdown in the mechanical interrelation of cells and blood vessels peculiar to the normal animal. It may be the normal outcome of a proper disappearance of a part of the normal blood supply which may result from the action of any substance or condition causing the formation of a local dense mass of cells in any part or tissues where conditions are not suitable for this mass to develop intercellular substances and a vascular network sufficient to remove quickly the stimulating product of the normal metabolism of the cell.

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Ribbert,[2] many years ago, appreciated that differentiation at least as far as it takes place in most organs and tissues of the body, does not affect in any way the growth of the cells. As he states, the remarkable power for tissues to regenerate after injury completely rules out an age factor in the cells determining their growth. The growth in cancer Ribbert did not find, therefore, unique in any sense. He thought it could be nothing more than the response that any cell may undergo with the proper change in environment.

The difficulty which confronted Ribbert and the school which has followed him has been to find the nature of the environment suitable for this change.

The recent work on coal tar and tissue culture has opened paths for such analyses. The theories of Yamagiwa and Ichikawa,[20] although not completely proven by them, are epoch-making in the history of the study of this disease.

Fischer,[2] in 1906, showed that when drops of olive oil containing Scharlach R are introduced just beneath the epidermis they cause a rapid migration of epithelial cells to them. These cells surround the drops like a collar. Later they proliferate and form a dense mass not unlike true cancer. Yamagiwa and Ischikawa later showed that if coal tar be applied frequently over a long enough time to one point on the skin of a rabbit these masses may reach a large size and metastasize like cancer. Woglom and Murray,[27] repeating their experiments, produced similar metastasizing tumors in mice. These tumors they found, when once established, could be transplanted through several generations of rats. The experiments have now been repeated by many others. One of the later series of experiments showed that this reaction takes place only about drops of tar having a high boiling point.

Ross[28] pointed out that those substances which in man have been shown to lead to cancer, such as paraffin, soot, coal tar, manure, etc., are active stimulants for the growth of cells. A more careful study by us of the action of many substances which lead to cancer has shown definitely that they lead either directly or indirectly to the production of a local dense mass of cells largely free from intercellular substance and poor in blood vessels.[1]

Jorstad,[34] working in my laboratory, has found that when a drop of coal tar is introduced on the surface of the skin or directly beneath the epidermis it occasions a movement of the epithelial cells toward it, a crowding, a primary degeneration, then an active proliferation of these cells

and the formation of a dense mass. The action of a single drop of coal tar is limited. If more be added, more degeneration, movement and proliferation result. Finally an independent dense mass of growing epithelium is established. If the same coal tar be introduced into the subcutaneous tissue of an adult it occasions a movement of connective tissue cells to it, a degeneration of the cells and hyalinization of the tissue. If it be introduced into a more cellular connective tissue like that peculiar to the embryo and younger individuals, large numbers of cells are drawn away from their blood vessels and intercellular material and collected in a dense mass about the tar. In this dense mass these cells then begin to proliferate actively. The same is true for other lipoid solvents.

In the same manner the spirotera of Febiger[29] attracts cells. This parasite induces cancer in the stomach of rats.

On several occasions I have also encountered the larva of the cat tape worm in the livers of rats. This larva has been shown by Bullock and Curtis[30] to induce sarcomata in the liver of rats. The capsules formed by the liver tissues about this larva are strikingly different from those induced in man about the ecchinococcus, for instance. About the former larva the capsule is a mass of connective tissue cells free from intercellular substances. About the latter the capsule is a dense fibrous mass. Sarcomata do not develop about the ecchinococcus cysts.

These substances act, therefore, quite differently from those which produce inflammation. These latter substances produce primarily an exudate from the blood vessels. The cells react secondarily to organize and become scattered in the exudate. The cancer-producing substances act on the cells directly to attract them. The tissue built in the former case is a richly vascular tissue with a large bulk of intercellular substance. In the latter instance it is a densely cellular tissue poor in intercellular substance and in blood vessels.

Ross pointed out that it is not injury that produces cancer. It is the action of specific substances which induce the proliferation of cells. It is neither, according to our studies. It is the action of substances which attract cells to them, away from their intercellular material and blood vessels, so that there is formed a dense local mass free from ready avenues of escape for the soluble products of the normal metabolism of the cell.

A soluble stimulus placed in the tissue can have no such effect. It must escape by way of the blood stream. It is a viscid mass, which remains fixed and attracts cells to it, that acts this way, such as certain animal parasites; thick drops of coal tar, drops of olive oil, or better, olive oil containing substances like Scharlach R.

The exudates of chronic inflammatory processes attract cells to them, as Hertzler's studies have indicated, and the work of cultures has proven. In the body the same conditions are associated also with the development of blood vessels and the exudates become coagulated and deposited as extracellular fibrils between the cells. Primarily, these processes lead away rather than toward any cancerous process. The epithelial cells of the glands and other parts do not react readily to these exudates. They become first imbedded in the mass of newly formed connective tissue. Later the mass of connective tissue changes. The blood vessels become obliterated. The connective tissue becomes hyaline and the cells degenerate. With this change in the blood supply the separated epithelial-lined ducts suffer atrophy to a greater or less extent. At the same time their environment becomes more suitable, therefore, for an active proliferation. Carcinoma is a disease of old age. It develops frequently in the chronic inflammatory process of the breast and other organs. It develops in the epithelial cells of the skin which are undergoing atrophy. As is well known, the breasts of women and probably other tissues suffer periodic stimulation from the sex glands and other internal secreting glands. As is evident from the above laws of growth laid down, such atrophying epithelium must respond quickly to any cellular growth stimulant, or as the culture experiments indicate, such a tissue may begin to grow independent of such stimulation. To prove this it became of interest to study the effect of a soluble stimulant upon tissues of adult animals. The stimulant used was the one produced by the tissue as noted above. It was extracted from the Jensen sarcoma with normal saline solution. The solutions of this stimulant were made sterile of sarcoma cells by passing them through a Berkefeldt filter. When introduced into the normal skin of an adult rat this solution has no noticeable effect. The stimulating substance is evidently removed by the blood stream. When injected into areas of skin suffering pressure atrophy this solution produces true carcinomatous proliferation.[20]

We have not investigated as yet the action of x-ray and radium. Members of families of mice which frequently develop cancer have also not been investigated. From the above observations it seems evident, however, that cancer may result from any condition or substance which can build a dense mass of cell in the organism, or from a sufficient reduction in the blood supply of any cellular tissue so that the growth stimulus may accumulate and the nutrition remain unchanged. The simple pigmented mole is such a mass of cells. These moles may become malignant after injury or disturbances in their blood supply or in the course of their normal de-

velopment in the aging organism. Such age changes are associated with a decreased vascularity and atrophy of the superficial tissue. The relation between senile keratoses and cancer has long been recognized by dermatologists.

Further studies of the mechanism of cell division which have just been completed in the laboratory show that an orderly division of cells to form columns and to line the ducts of glands is directly related to the circulation and position of the blood vessels. Decrease this circulation and stimulate the cells they divide so as not to increase the circumference of the duct, but to fill the duct with cells. Thus dense masses of cells develop.

While it is true that many authors, such as Child,[36] have assumed that differentiation is the normal outcome of all cellular growth, it has been possible to prove very definitely that this is not true. The conditions which lead to a primary laying down of intercellular substances and a growth of blood vessels is something peculiar to the body and not the immediate process of growth in the cell. These changes are not the result of growth, but they stop the growth of the cell in the developing organism. In the fully developed organism and even evidently at a much earlier period this act becomes secondary, like growth, to specific stimuli. In all regressing tumors, as previous authors have shown, the cells do not degenerate. A general increase in intercellular substance, a fibrosis, marks the end of growth in many of them.[2]

Hertler,[32] in 1910, gave proof to show that the connective tissue fibrils are not secretion products of the cell, but simply transformed fibrinogen of the exudate of wounds. The tissue culture has shown very definitely that the fibrinogen acts as a special stimulant for the growth of endothelial cells of the blood vessels and migration of connective tissue cells. These cells coagulate the fibrinogen to fibrin and cling to it. There is no evidence that the connective tissue cells secrete fibrils at any time.[8] This fibrin is transformed into the extracellular connective tissue fibrils.

Such extracellular formations develop, however, only in areas where the stimulating substance noted above is in low concentration. Where it is formed in greater amounts the fibrin is digested. This stimulating substance, as I have noted above, is a product of oxydation in the cell. It acts according to its concentration. This concentration may be varied by preventing or accelerating its escape from the cells or by cutting down the oxidizable substances so less is formed.

As many of the earlier studies of development have indicated, this laying down of intercellular substances and differentiation in general is directly associated with the decrease in the yolk supply. If you cut the yolk into two, fully differentiated animals of half the size and with half the normal number of cells are produced. Gudernatsch[35] showed that if tadpoles are fed thyroid they will differentiate in a few days. Thyroid leads to a great increase in the metabolism and the burning of nutrient materials in the body.

The striking peculiarity of body cells which distinguishes them from the unicellular organism is not only their general organization which has to do with their growth and function, but their inability to take in crude materials from Nature and utilize them as food. These must first pass through a fully formed body.

The developing animal depends for its nutrition on the yolk and the mother. The yolk is formed by the parent body. In man, as in all higher animals, all the essential organs are formed before it is possible to remove the animal from its yolk or from its mother.

All my attempts to feed these body cells artificially, like bacteria, have failed. Extracts of embryos rich in yolk is the only food I have found capable of nourishing them.[33]

In a certain number of individuals, fibrosis becomes a striking feature of the later life. It is interesting that cancers do not develop actively in these individuals, but they tend to develop intercellular substance. It is in the young healthy person that cancer quickly spreads beyond the bounds of the surgeon's knife and destroys quickly. It is in young animals that transplants of cancer take readily.

Any dense mass of cells once established either through a primary atrophy or secondary stimulation must tend in the normal organism to reproduce itself. This mass becomes parasitic to the host and continues in its growth to the destruction of the whole. Morgan[37] several years ago showed that an actively growing tissue may take its nutrition directly from the differentiated cells of the organism. Legs of salamanders regenerate as rapidly in starved as in well-fed animals. The starved animals, regenerating legs, suffer extreme emaciation and atrophy of their organs. In the normal organism an actively growing mass must receive ample food, produce ample of the stimulating substance and thus continue to reproduce itself.

The cause of cancer may be, therefore, any substance or condition which can primarily build a dense mass of cells in the organism or cause a proper change in the blood supply to a cellular tissue. As early as 1913, I noted the direct relation of the number of cells per unit area in the fragment to their ability to grow in the cultures. Later, just at the beginning and after the War, I noted the relation of growth in cultures to a decreased circulation and the importance of oxy-

gen for the development of the growth stimulus. Unfortunately, three years ago, in beginning to work directly with cancer, I became misled by a possible parasitic explanation of this disease. All experiments destined to prove such a parasitic origin have forced me back, however, to the conclusions cited above. It was only after these more direct experiments had progressed, however, that I realized that a very plausible explanation of this disease was in our hands.[1]

By these observations it has been possible to show, therefore, that an autonomous growth of cell is not the result of any primary change in the cell, but the direct result of a crowding of the cells and an orderly decrease in their blood supply. The stimulus for growth, other than food and oxygen, so well recognized by the earlier students of regeneration and cancer, is a product of the normal metabolism of the cell. The cancer cell is not different from normal cells. It is a normal cell reacting to a new arrangement of cells in the body. Cancer, therefore, for the first time becomes explainable in terms of the facts as they exist. To cure this disease this mass must be removed or destroyed, as it is today, or we must find some peculiarity in the growth reaction of these cells to attack. Its cure is not going to be solved by the application of the general laws which have held for infectious diseases, but through further careful analyses of the finer details of cellular structure and metabolism.

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DISCUSSION

Dr. J. A. Lanford, New Orleans, La.—The most interesting thing that I gathered from Dr. Burrows' paper was the conditions that he found would influence or occasion proliferation of embryonal cells as well as misplaced cells in the living body. I find it, though, rather difficult to understand why, if these cells are shut off from their circulating medium, they should get into the systemic circulation and produce secondary growths in distant parts of the body.

Another point that was shown with his tissue culture is that ordinary heart muscle could undergo a metaplastic change producing connective tissue and vice versa. This would tend to em-

phasize the fact that there is inherent in the embryonic cell some of the properties of the primary cell from which the tissue is derived, that it might under certain conditions produce a connective tissue, and under other conditions a muscular structure or any other type of tissue of that group.

Carrel and Burrows showed that the plasma from a rat which is harboring a new growth (sarcoma) is a more preferable medium for the culture or growth of that sarcomatous tissue *in vitro* than the plasma from a healthy rat of the same species. The inference is that there must be something in the individual's plasma that tends to stimulate the growth of these embryonal types of cells.

Dr. Charles H. McCollum, Fort Worth, Tex.—We must believe that there is something peculiar about tumor cells which is not entirely dependent upon the influence of the body of the host since they grow with much vigor when they are dislodged from their place of origin and may even be transplanted and grow in another host. It is difficult to believe that any mechanical massing of cells will bring about these and other extraordinary peculiarities with which we are familiar in the case of malignant tumors as contrasted with benign ones. That there may be great changes in the susceptibility of the host is equally well known, especially for the recent work on the effects of tar.

But many things lead one to the belief that the whole mode of growth and multiplication is morphologically and functionally different from that of normal tissues and the immunity reactions which can be produced by such growths against further transplantation serve to strengthen this belief.

A very interesting part of Dr. Burrows' paper was his discussion of stimulating substances which affect the metabolism of cells and are possibly concentrated about an implantation forming its growth. This seems a very promising line of investigation.

A Member.—It seems to me that these cells that are cultivated are not connective tissue cells, but cells which have been arrested in their differentiation and they have lost under surrounding conditions the property of going on and completing differentiation. Under the conditions which exist they are able only to perform functions in relation to themselves and not in relation to the body as a whole.

I have been comparing the tumors of men with the enlargements on trees, the so-called cedar growths, and I have been making some cultures. I have questioned whether any malignant tumors or any benign tumors, fibromata or adeno-cystomata, have a parasitic basis. In the case of the coal tar product an irritant is applied and you have to continue to apply the irritant to have the condition continued. It does not metastasize, and I question whether it could be considered a neoplastic disease. If these tumors are parasitic, either we have not recognized the parasite or we do not understand it and its life history. I question whether you can consider these cells connective tissue cells in the culture. They are cells which have been arrested in their differentiation.

Dr. S. T. Darling, International Health Board, New York, N. Y.—In regard to the question of the proliferation of metazoal cells as being due to an over-stimulation or the removal of some repressive substance, I should like to refer to an observation of mine made some years ago while studying a strain of Entamoeba histolytica. This strain had been carried along in kittens and after several removes bizarre changes were noted in the nucleus of the cells. These changes had been erroneously interpreted by Schaudinn as developmental in origin, but I was impressed with their resemblance to appearances seen in the nucleus of cells in malignant neoplasms, for there were evidences of rapid multiplication and degeneration. Here, either an unusual rate of multiplication due to stimulation, or unrestrained activities, brought about a condition analogous to a neoplasm.

Dr. Burrows (closing).—In my earlier studies I noted that the fixed tissue cells elaborate a substance during their migration which is an active blood coagulant. The whole movement of these cells is exactly proportional to the adsorption of such a substance by the medium. The action of this substance is manifested about fragments of mesenchyme of older embryos and fragments of connective tissue of adults. It does not appear at once, but after a latent period of several hours. It is manifested at its appearance by an active contraction of the plasma clot or by the formation of fibrin and serum. The mesenchyme and connective tissue cells move out in contact with the fibrin as it forms. This contraction continues to spread outward for a time, then ceases and with it the cells cease to move further.

When one uses fragments in which the cells are more densely packed as similar fragments of younger embryos, fragments of cellular granulation tissue or sarcomata this contraction commences earlier. It is more extensive and a film also forms along the surface of the medium. The cells move first into the clot and then in this surface film. They leave the clot for the film on the surface of the medium. Later growth and division intervenes.

The plasma itself does not supply any nutrition for the growth of the cells or the necessary energy producing substances for the migration of these cells. The cells during migration lose materials and become exhausted. They supply their own energy for migration. The border cells of these more cellular fragments will migrate and grow in a nutrient free salt solution. The cells move out in the surface film, which covers the medium and makes it leathery. Nutrition comes, as I pointed out in previous articles, from disintegrating cells in the center of the fragment. This degeneration is not an autolysis in the true sense of the word. The degeneration without oxygen does not liberate nutritive sub-

stances. Nutrition is liberated from cells degenerating in a cellular fragment of sufficiently small size to allow oxygen to diffuse to all its parts. This degeneration is the result apparently of the accumulation of certain products of the normal metabolism of the cells. A normal salt solution extract of these tissues will cause in high concentration a degeneration of cells which lead to the production of nutrient substances. Growth synthesis like this splitting of nutrient substances depends again on a stagnation and concentration of normal products of the metabolism of these cells. The cells at the border of these cellular fragments are at first carried out into the medium as the cells degenerate in the center of the medium. As the medium becomes more saturated they grow and divide and then later also degenerate if they are not removed to a fresh medium. This degeneration may also be delayed by washing the culture with serum.

The question of metastases resolves itself around the question of the conditions which regulate migrations. It is interesting to note that in these cultures migration ceases in those areas where active growth intervenes. In the less cellular fragments or fragments having less concentration of the stimulating products of the metabolism migration alone intervenes. As the products of the metabolism accumulate in the cultures of the more cellular fragments the cells in the outer medium cease to liberate the coagulating substance and migrate. They grow and divide at the expense of the surface films from degenerating cells in the fragment. Whether growth or migration intervenes is wholly quantitative as far as the cultural experiments indicate. Degeneration like that seen in cancer of a non-toxic type is also merely the result of a greater increase in those products necessary for normal digestion and synthesis.

Through oxidation these cells liberate activators or substances which are necessary for the breaking up of food and synthesizing it. The isolated cells cannot retain these in concentration sufficient to perform these acts. They must accumulate about the cells. They are washed away by an active circulation. Stagnation and crowding is essential for them to grow. This is true also, as I pointed out, for unicellular organism, but not to the extent that it is true for body cells. Too great stagnation leads to the digestion of the cell itself.

The action of coal tar I have explained as the result of the absorption of it by the substances which lead to the migration of the cells. In recently published experiments we have found that large doses of coal tar lead to cachexia and death like cancer. Old masses of coal tar in the tissue also eventually migrate or metastasize by way of the veins to distant organs. Coal tar as we have seen it does not stimulate growth necessarily, but attracts cells to it away from their intercellular substances and blood vessels and forms dense masses which are suitable for growth to intervene.

Cancer cells in culture are not capable of so great a growth as embryonal cells. The embryonal cells are apparently richer in nutrient materials. Extract of cancer stimulates growth, but does not prolong it. Carrel has shown that the juice of embryos will prolong growth indefinitely in the cultures. Drew has failed to get any such prolongation of the life of the cells with the juice of cancerous tissue, and I have had the same experience.

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Relation of oxygen to the growth of tissue cells. MONTROSE T. BURROWS.

It has been found that normal digestion, syntheses as well as migration of body cells, depends on the accumulation about them of certain blood-soluble products formed by them only in the presence of oxygen. Autolytic products are toxic. The stimulating products are formed only in the presence of oxygen and their formation is proportional to the oxygen present. This stimulating substance or substances in high concentration causes a digestion of the cells together with proteins and fats in the culture medium. In lower concentration fats disappear in the medium, the cells grow and divide. In still lower concentration migration alone and function (rhythmical muscular contraction) is induced. The cells digested in the presence of a high concentration of this stimulating substance or substances are food for other cells. These products in high concentration cause a digestion of the cells histologically similar to autolysis resulting from the absence of oxygen. This stimulus or stimuli may be readily washed away in the culture. The actively growing embryonic tissues, the tissues of cancer, wounds and the nail, have a poor circulation. The circulation in these tissues in either sinusoidal or the capillaries are few in number per unit cell area. A study of the substances and conditions which lead to cancer, such as coal tar, other lipoid solvents, certain congenital tumors, chronic inflammation, lead either directly or indirectly to build conditions suitable for such an accumulation of the growth stimulus about the cells. They build a densely cellular tissue relatively poor in blood vessels or a tissue in which the capillaries are dilated and the circulation within them is sluggish. Studies of tissue cultures have shown that stagnation and crowding leads to growth. Scattered cells and cells well washed are preserved and become inactive.

"SOME NEW LINES OF PROGRESS IN CANCER RESEARCH"

To the Editor:—The editorial entitled "Some New Lines of Progress in Cancer Research" (THE JOURNAL, January 5) discusses an article by Dr. J. A. Murray, director of the Imperial Cancer Research Laboratories of London, England. This article appeared in the *Lancet*, July 28, 1923. Murray attempts to show that cancer is the result of cell injury, that it is a degenerative overgrowth somewhat perhaps in the sense of Thiersch and Benicke and later of Oertel. The growth in cancer he attributes to the presence of a growth stimulus liberated when cells are injured. He cites work with the tissue culture as proof for this deduction. What I wish to point out here is that I do not believe that the work with the tissue cultures carried on in this country justifies this deduction. In Murray's laboratory, Drew has shown that embryonic tissue and malignant tumor tissue contain an extractable growth stimulus. Drew has again found this stimulus in kidney left in the incubator for one hour. Extracts of fresh kidney in the cold showed no such stimulating substance. Drew and Murray ascribe the presence of the stimulus in the incubated kidney to autolysis. Autolysis is self-digestion resulting from any tissue injury that disturbs the normal oxygen supply. One hour is not sufficient for true autolysis to take place.

Carrel and I, as early as 1911 (THE JOURNAL, Jan. 7, 1911, p. 32), had noted an active stimulus in these tissues. While Carrel has stated in later articles that stimulating substances are to be found in many adult organs and tissues, I have not been able to confirm his experiments. As I pointed out in 1913, any dilution of the plasma which Carrel used as a medium leads to an increase in the rate of migration and growth. Carrel had failed to control for such dilution. Controlling these factors carefully, it can be shown that active stimuli sufficient in quantity to induce a migration and growth of cells are peculiar alone to actively growing tissue. Such stimuli for growth and migration of cells do not exist in fresh pieces of adult organs and tissue or in the tissue of older embryos. These stimuli develop, however, in these older tissues when they are removed from the body and from their normal circulation but not from their oxygen supply. As early as 1913 (*Tr. Cong. Am. Phys. & Surg.* **9**:77, 1913; *Tr.*

XVII Internat. Cong. Med., Gen. Path. & Path. Anat., London, 1913, p. 217) I had shown that oxygen diffuses only from 0.5 to 0.7 mm. into clots of plasma in quantities sufficient to maintain the oxyhemoglobin in red cells. Into tissue it diffuses somewhat more, but rarely more than from 1 to 1.5 mm. Fragments larger than this size suffer central autolysis. This autolysis liberates products that are distinctly harmful for growth. Again, in 1913, I gave evidence to show that the rate of formation of this growth stimulating substance is proportional to the number of cells present in the fragment and within the range of oxygen diffusion. In later experiments (*Proc. Soc. Exper. Biol. & Med.* **18**:133, 1921), I again showed not only that oxygen is necessary for the development of this stimulus but also that the amount of stimulus formed is directly proportional to the amount of oxygen present. At no time, however, need the quantity of oxygen be large in amount. Fletcher, several years ago, had shown that muscular contraction may proceed without oxygen. My experiments show that growth may likewise proceed without oxygen when the stimulus is present. It fails in the absence of oxygen when the stimulus is absent. The stimulus can be formed only in the presence of oxygen. There is no evidence whatsoever that this growth stimulus is a product of cell injury. It is a normal product of the cell's metabolism. In high concentration it causes a digestion of the cell. Extracts of these digested cells are food for other cells. In slightly lower concentrations it induces a growth of the cells; at this and higher concentrations the fats as well as proteins are digested in the cultures (Burrows: four papers under heading of Studies on Cancer, *Proc. Soc. Exper. Biol. & Med.* **21**:94-110, 1923; *Missouri State M. J.* **20**:145, 1923).

As I have further shown in these later papers, this stimulating substance is soluble in physiologic sodium chlorid solution and in serum. Growth in the cultures can be inhibited by washing the cells with serum. Actively growing tissues in the body are characterized by a relatively poor blood supply. Either the capillaries are few per unit cell area, or the circulation is sinusoidal in character. This is true for the nail, the wound, granulation tissue, embryonic tissues and for cancer.

Cellular growth, as shown definitely by the cultures, depends on the accumulation of a certain normal product or products of the cell's metabolism. This product or these products are soluble in circulating body fluids. Growth may be inhibited by decreasing the number of cells per unit area or by increasing the vascular supply; in other words, by

decreasing the amount of production of the stimulating substance or by washing it away as it is formed. This fact I have found to have quantitative expression in the culture.

The fact that the adult cells, when cut off from their vascular supply and not from their oxygen, may grow in a tissue culture indicates clearly that the important factor which stops growth in the developing animal is the development of intercellular substances and a rich vascular supply. There is no evidence, as Ribbert has clearly pointed out, that this cessation of growth is due to any aging of the cell.

In the light of these facts, cancer may be readily deduced as nothing more therefore than the restoration in any part of the body of those conditions necessary for the cells to grow. In other words, it may result from any condition or substance capable of inducing the development of a dense mass of cells having a relatively poor circulation—conditions suitable for the stimulating substances to concentrate (Burrows: *Tr. Sect. Path. & Phys.*, A. M. A., 1923).

As I have stated above, a low concentration of the stimulus causes migration. It induces the cells to liberate a substance that is an active blood coagulant. This substance decreases the surface tension of cells in the presence of fibrinogen. They migrate along the lines of diffusion of this substance into a plasma clot (Burrows, 1913). Active blood coagulants extractable from these cells are phospholipins, according to Woolbridge and Mills. They are very soluble in certain coal tar products. Mr. Jorstad, working in my laboratory, has shown that coal tar acts to produce cancer in that it attracts cells to it as the fibrinogen attracts them. It thus attracts the cells away from their normal vascular supply and forms dense cell masses suitable for independent growth. The coal tar does not stimulate growth directly (*Proc. Soc. Exper. Biol. & Med.* **21**:67, 1923). I have found that chronic inflammation, various animal parasites, and congenital tumors lead either directly or indirectly to the same formations (Burrows: Studies in Cancer, *loc. cit.;* paper read before the Southern Medical Association, Washington, D. C., Nov. 15, 1923).

Murray's conclusions are, I believe, unjustifiable in terms of any careful analysis of the facts gleaned from the study of body cells grown in vitro, and are out of accord with many of the best biologic facts deduced recently and in older times. Wildiers, in 1902, had already shown that single yeast cells cannot grow in a large bulk of medium. In 1913, I noted that the rate of growth in a tissue culture is proportional to the size and cell density of the fragment, when the size

of the fragment is kept within the limits of an active oxygen diffusion. This I have again pointed out clearly in recent articles. Robertson found the same to be true for the growth of paramecium, and in a recent book he has shown that the growth curves of the body are not regular but similar to a reaction suffering autocatalysis.

From the work on the tissue culture, it is evident that growth of body cells depends always on the accumulation of certain soluble products of their metabolism about them. These substances are formed in the oxidation reaction. Cancer need not be considered a degenerative phenomenon. If one is to give an explanation for cancer from the studies of tissue cultures alone, it must be assumed to be the result of a condition capable of establishing a dense mass of cells having a relatively poor blood supply (conditions suitable for maintaining a certain high concentration of certain blood soluble oxidation products about the cells). Our studies of coal tar, other lipoid solvents, chronic inflammation, certain congenital tumors, animal parasites, etc., have confirmed this view.

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Tissue growth and vitamines. MONTROSE T. BURROWS, Barnard Free Skin and Cancer Hospital and the Department of Surgery, Washington University School of Medicine, St. Louis.

The B. tumefaciens when injected into the tissues stimulates an active growth of cells. These cells grow to form a dense, non-vascular cancerous tissue. Jorstad and I have tried the effect of feeding this organism. It acts as a ready and even more efficient substitute for vitamin B (yeast) in a diet containing no vitamin B. The growth stimulus of this organism allowed to act through the blood vessels produces a normal vascular and functioning tissue or a normal growth of the animal. Acting outside of the blood vessels it produces in contact with the tissue cells a cancerous organization. The two day old cultures which we have tested contain no detectable vitamin A.

STUDIES ON WOUND HEALING*

I. "FIRST INTENTION" HEALING OF OPEN WOUNDS AND THE NATURE OF THE GROWTH STIMULUS IN THE WOUND AND CANCER

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While it has been known since the earlier work of Peters, Nussbaum and others [1] that the migration of fixed tissue cells plays an important part in the healing of wounds, the general relation of this process to the growth of the cells and the conditions leading to the gross contraction of the wound remain as yet obscure. The relation of the various cells concerned to the end result is also an unsolved problem.

Most authors have looked at the healing of skin wounds as the result of a primary overgrowth of epithelial and connective tissue cells and a secondary contraction of these cellular masses or the cicatrix. In infected granulating wounds the connective tissue overgrowth is excessive. These infected wounds first fill with granulation tissue. Later as the infection is removed the epithelial membrane of the surrounding skin then spreads over the granulation tissue. With this later growth of the epithelial cells the granulation tissue suffers shrinkage, through atrophy and hyalinization, and pulls the old edges of the wound closer together. In clean wounds the picture differs in that the growth of the granulations is not so excessive. L. Loeb and his students [2] concluded from a study of the healing of wounds of the skin of the ears of guinea pigs and rats that the changes are a primary migration of epithelial cells as a membrane from the edges of the wound and a growth of connective tissue from deeper parts. Contraction is the last act and results from a later regression of this cicatrix.

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From observations on many healing skin wounds in the clinic I concluded that clean wounds may also heal somewhat differently. Many of these wounds heal by an early contraction of the edge without the appearance of any epithelial membrane or noticeable granulation tissue from beneath. In the former cases the contraction is evidently due to a secondary contraction of the overgrowths. In this latter case it is a primary phenomenon preceding and holding in check the growth of the connective tissue cell. A large part of the wound is thus almost completely covered by the stretched skin of the edges without any gross evidence of the formation of new tissue.

While it has been known for many years that open wounds may heal by 'first intention' the nature of the conditions which lead to this more ideal type of healing have not been determined. It was hoped by a more careful study of the behavior of connective tissue and epithelial cells in the tissue culture and by a further comparison between the reaction of these cells in the cultures and in the wound that it might be possible not only to learn more about the nature of the conditions which control these various types of healing but also to deduce something about the nature of the stimulus which leads to the growth of cells in the body after injury.

In the granulating wound the growth of the epithelial cells is delayed. The granulations grow excessively. In the simple wounds the growing epithelial cells inhibit apparently the growth of the granulation tissue. Thiersch and Remak had noted evidence of an antagonism between epithelial and connective tissue cells during development and in cancer. This antagonism had not been resolved, however, to understandable and practical terms.

In previous articles [7, 11] I have pointed out that the active stimulus for the growth of cells in the tissue culture other than a proper temperature, food and oxygen, is a crowding of the cells together in a stagnant environment. I had also related these factors of stagnation and crowding to the stopping of growth at maturity, to hypertrophy and hyperplasia, and to cancer. I had shown that the conditions and substances (such as chronic inflammation, coal tar, etc.) which lead to cancer act

only to bring cells together into a compact mass and reduce the blood supply to this mass. They do not induce growth. Growth is the result of this primary crowding and stagnation.

Whether the same conditions are responsible for growth in the wound I had not determined. I had also not explained how a cancerous organization thus induced by coal tar or other substances or conditions can continue to grow to the destruction of the body and independent of the primary conditions or substances which had induced it.

The Behavior of Connective Tissue and Epithelial Cells in the Cultures

In cultures of fragments of skin where blood plasma is used as the medium I have noticed that the growing epithelial cells antagonize the growth of the connective tissue cells about fragments of adult skin while the reverse is true for the growth from fragments of embryonic tissue. The epithelial cells of the skin rarely ever separate from each other in the culture. They move out generally as membranes from the fragments, Fig. 7. The connective tissue cells on the other hand separate themselves readily from the fragments and become dispersed in the medium.

Connective tissue cells rarely ever migrate from fragments of the whole adult skin. They migrate only from fragments of the dermis and superficial fascia from which the epithelial layer has been removed. The epithelial cells not only begin their migration after a much shorter latent period but they also bring about conditions which completely inhibit connective tissue cell activity within and about these fragments of adult tissue. What is true for adult skin is not true however for that of the embryo. In embryonic life the mesenchyme is a densely cellular syncytium. The epithelium is only a double layer of cells. About fragments of this tissue placed in the cultures the mesenchyme cells migrate out after a very short latent period. While in a few cases the epithelial cells may migrate with them, in most cases these epithelial cells degenerate or lose their substance to the growing mesenchyme.

As development proceeds, the epithelial layer gradually thickens and the mesenchyme cells decrease per unit area in the tissue. The epithelial tissue remains a densely compact layer of cells, while the connective tissue cells become widely separated by the laying down of the intercellular fibres. As these changes proceed the epithelial cells gradually dominate over the connective tissue or mesenchyme cells. They migrate quickly from adult fragments placed in the culture while the migration of the connective tissue cell, rapid in early life, becomes slower and slower with these age changes and the latent period before

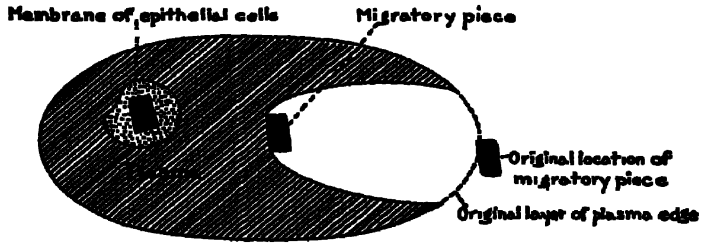

Fig. 1. A diagram to show the behavior of fragments of frog's skin when embedded in the clot or placed against its edge. The original position of the piece placed against the edge is shown in outline. This piece has migrated into the clot and dissolved a wide area of the clot along its sides and behind it. In the left part of the layer of plasma a similar piece of skin embedded in the clot is shown surrounded by its stretched epithelial membrane.

these cells begin to migrate from fragments placed in the culture becomes greatly prolonged.

In this regard the response of these cells in the culture is like that for pathological growths in the organism. Sarcoma is a disease more peculiar to early life while carcinoma is more common in later periods. The question arose how important is this dominance of the epithelial cells in the healing of wounds.

In 1913,[3] in attempting to ascertain the mechanism of the formation of epithelial membranes and how they prevent the migration of the connective tissue cells from the fragments of skin of older embryos and adults, I further noted that these epithelial membranes never form about fragments of skin except when both the fragments and the plasma clot are firmly anchored. When the plasma clot is unattached it is drawn in

mass to these fragments and dissolved. When the fragments are not anchored they migrate in mass long distances into the plasma clot. No epithelial membranes appear from these fragments during their migration as none appears while a loose clot is being pulled to an anchored fragment and dissolved.

At the same time [3] I also studied the mechanism of the formation of these epithelial membranes and the healing of simple skin wounds cut through the middle of fragments of skin which were placed in the plasmatic medium of the culture. These epithelial membranes which form about the fragments are the original stretched membrane of epithelial cells which cover these fragments. This stretching is either the result of an active contraction of the clot to which the border cells of the membrane are attached (Experiment K_6 No. 7, Fig. 2) or to an actual migration of these border cells over the surface of a contracted clot and an active dissolution of the clot behind them (Experiment R_4 No. 6, Fig. 3).

Experiment K_6 No. 7. In this experiment I placed a fragment of the skin of an 18-day old chick embryo one millimeter thick, in a layer of plasma according to the technic described elsewhere.[4] The plasma clotted quickly after the tissue fragment was added. Along one side of the fragment the plasma loosened from the connective tissue but remained attached to the border cells of the epithelial membrane. This part of the clot suffered an excessive contraction. In contracting against its firmer attachment to the cover glass it stretched the membrane of the fragment. The fragment was held in place through its attachment to the clot on its other sides, Fig. 6.

Experiment No. 6 R_4. A fragment of skin and connective tissue covering the anterior lymph sac of a frog was removed and the endothelial lining of the lymph sac scraped away. The piece was then placed in a layer of plasma 0.5 mm. in thickness spread on the surface of a cover glass. The plasma was prepared from the blood of an adult frog. As soon as the tissue was laid in place a hollow slide rimmed with vaseline was placed over the cover glass. The culture was thus sealed in a small moist air chamber. After a few minutes the plasma clotted. The slide was then turned over so it could be observed readily

under the microscope. The whole procedure was carried on and the slide was kept in a room heated to a constant temperature of 70° F.

After one hour an epithelial membrane began to stretch out from all sides of the fragment into the medium. Just preceding this movement the plasma clot underwent marked contraction with the formation of fibrin and serum. The cells at the edge of the membrane clung tightly to the fibrin thus formed and moved outward over it. Directly behind these outer border cells the plasma underwent a rapid dissolution. This dissolution involved at first only a part of the clot immediately behind the outer border cells. Slightly farther back it extended downward through the whole layer. That part of the membrane between its border cells and the fragment thus became entirely free of any solid attachment, Fig. 7.

In all such cultures this movement of the outer border cells continues only to the limits of the elasticity of the membrane. The membrane may then tear loose from the clot or breaks in the middle. The freed parts retract against their points of remaining attachment. If one cuts this membrane during its formation it retracts immediately. If one loosens the fragment it is drawn against the clot through the retraction of the elastic and stretched membrane. As is evident, such forward movement of the border epithelial cells which stretch this membrane must drag an unattached fragment or skin edge with it.

In other experiments I cut small button holes through larger but similar fragments of frog skin. I placed these fragments in layers of plasma prepared from the blood of adult frogs. These button holes or wounds become covered by the epithelial membrane when their edges are well anchored. When their edges are freely mobile they are drawn together by the moving epithelial membranes, Fig. 2.

In these wounds cut in pieces of adult frog skin of the culture, one never sees any connective tissue cell activity. The migration of the connective tissue cells is not only temporarily but permanently inhibited. The mobile connective tissue edges are also never drawn completely together.

At first I thought that this failure of the connective tissue

cells to move out from these fragments might be due only to the liquefaction of the clot. Harrison in his earlier studies had noted that body cells cannot move into a liquid medium. They move only along fibrin fibrils or surfaces of the medium. A careful study of serial sections of these fragments has shown

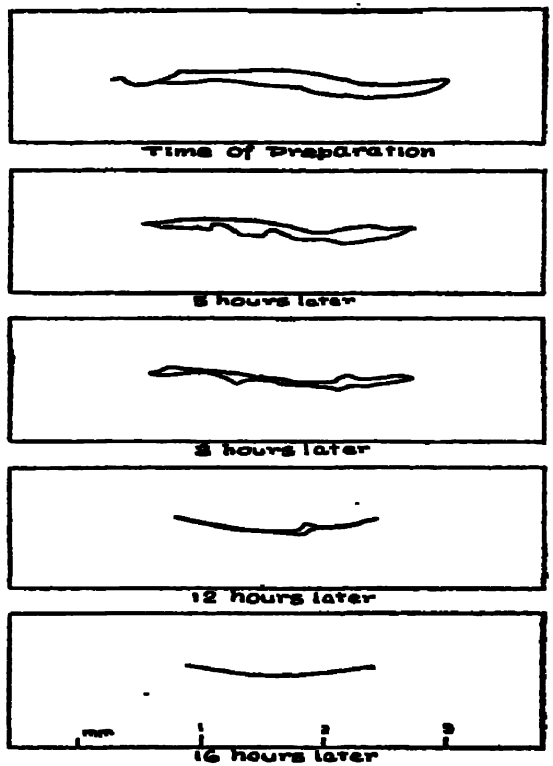

Fig. 2. Camera lucida drawings made at stated intervals of the edges of a healing buttonhole cut in a fragment of frog skin which is embedded in a hanging layer of plasma.

however that other inhibiting changes also take place. The connective tissue cells may be destroyed in these fragments. The epithelial cells in dissolving the clot not only destroy a path for the migration of the connective tissue cells but they may actually destroy the connective tissue cells themselves. In earlier studies [5] I had shown that the embryonal mesenchyme may migrate and grow at the expense of the epithelial

cells of the fragment. In these adult fragments of the frog skin the reverse is evidently true.

The adult connective tissue cells unlike the epithelial and embryonic mesenchyme do not commence to move two or three hours after the cultures are prepared but only after two or three days. They separate from the extracellular fibrils of the fragment to invade the clot. They are few in number. They cause no dissolution of the clot but transform it rapidly into course fibrils of fibrin along which they migrate. Quite unlike the epithelial cells these connective tissue cells never drag the fragments with them. I have studied the healing of buttonholes cut through fragments of pure mesenchyme and connective tissue. In fragments of young mesenchyme where the cells are a part of a syncytial network the edge of the buttonholes may be drawn together somewhat. As a rule, however, these edges only thin out as the cells leave them. In fragments of connective tissue the cells always leave the edge and invade the clot. They transform this clot into fibrin fibrils which radiate across the wound. In contracting, these fibrin fibrils pull the edges slightly together. For the most part the healing takes place by a filling up of the wound with migrating and growing connective tissue cells.

These facts are illustrated in Fig. 8 (*a* and *b*). These figures are drawings of a culture of a piece of the skin of an 18-day old chick embryo. A buttonhole had been cut through the skin and the epithelial membrane had been cut away from one side of the buttonhole. The sharply outlined edges of the buttonhole are the parts covered by epithelial cells. Fig. 8 (*a*) was drawn immediately after the culture was made. Fig. 8 (*b*) shows the condition of the cultures 30 hours later. Mesenchyme cells have migrated into the wound from the part which has no epithelial covering. This edge of the buttonhole has also remained more or less fixed as shown by the mark (*x*) on the cover glass. The part covered by epithelial cells, on the other hand, has shrunken, effecting a considerable closure. The mesenchyme cells have also migrated to a slight extent from beneath the epithelial layer. The activity of the more densely cellular mesenchyme of this embryonic skin is not so

completely inhibited by the epithelial cells as the connective tissue cells beneath the epithelium of the adult fragments.

Healing of Skin Wounds in Animals

In the light of these facts I had questioned, therefore, whether the primary outward extension of an epithelial membrane from the edge of a wound in the ears of animals, described by Loeb, might not be merely peculiar to skin wounds of those regions of the body where the skin is attached to deeper rigid structure. In other localities, such as in the loose skin of the trunk and extremities, it was possible that the picture might be different. Epithelial membranes had appeared only from firmly anchored fragments of skin in the cultures.

I had also asked the question whether the active contraction of the granulation tissue in the infected wound might not be due to a dominance and destruction of this tissue by the epithelial layer and whether this dominance as well as all growth in the wound might not be, as in the cultures, a response to stagnation resulting from the destruction of blood vessels and the inflammatory congestion rather than one of purpose for healing. The epithelial layer had become dominant in the cultures only where it was the more densely cellular tissue.

Method of Preparing and Studying the Wounds. The wounds studied have included only the skin and superficial fascia. Most of the wounds were made in the skin of the abdomen and back. A few were made in firmly anchored parts of the skin of the neck and head. Seventy rats and guinea pigs were used. The animals were given ether. The hair was removed with a 20 per cent solution of Na_2S. The skin was then cleaned with warm tap water and alcohol. The wounds were made by first cutting a circular incision with a cork borer. This incision was carried to the deep fascia. The piece thus isolated was lifted out and cut loose from the deep fascia with small curved scissors.

As soon as the wound was made, a sterile glass slide was laid over it and its edges carefully traced with a pencil on the glass. This outline was then transferred to a note book. At regular

intervals of 12 to 24 hours similar outlines were made of the old edges of the wound until closure was effected.

No dressings were placed on these wounds. The animals on the other hand were placed in cages on sterile towels. The towels were changed frequently until a scab had formed. Infections have at no time been troublesome. Foreign bodies

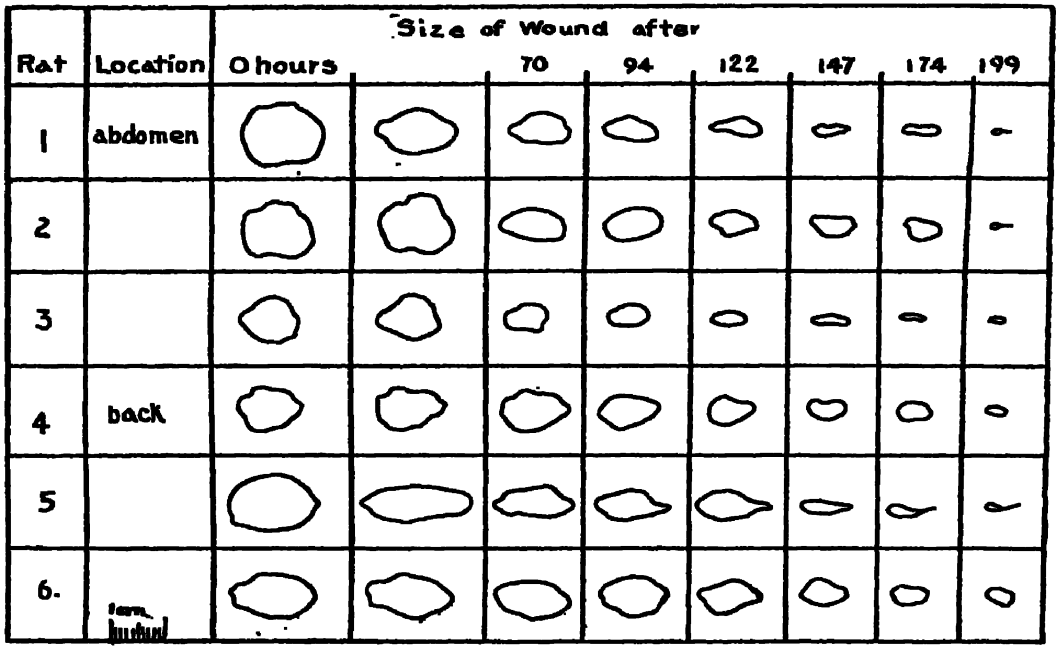

Fig. 3. Outline of original edge of wounds in the skin of the abdomen and back of rats showing how these edges have moved together.

were found in a few of the wounds and there was a moderate leucocytic reaction in a part of them. It was not the absolutely sterile wound that interested me: it was the mechanism of ordinary closure that seemed important for investigation.

In several of the wounds the scabs became very large. These large scabs not only slowed the healing but prevented the measurements of the edges of the wound. Such scabs were removed from time to time. They formed mostly on the wounds which had bled freely or were slightly infected.

Periodically during the experiment certain animals were killed with ether. A piece of skin containing the wound was spread on a cardboard and fixed in formalin. Blocks cut across

these wounds were sectioned and stained. The sections in each case were made perpendicular to the surface of the skin.

The cork borers used for making these wounds measured 10 and 12 mm. in diameter respectively. The freshly made wounds gapped frequently, however, to take larger dimensions and various shapes peculiar to their location. On the abdomen when the animal is stretched out on the operating table the wound gaps and assumes an ovoid shape with its long axis parallel to the long axis of the animal. When the anesthetized

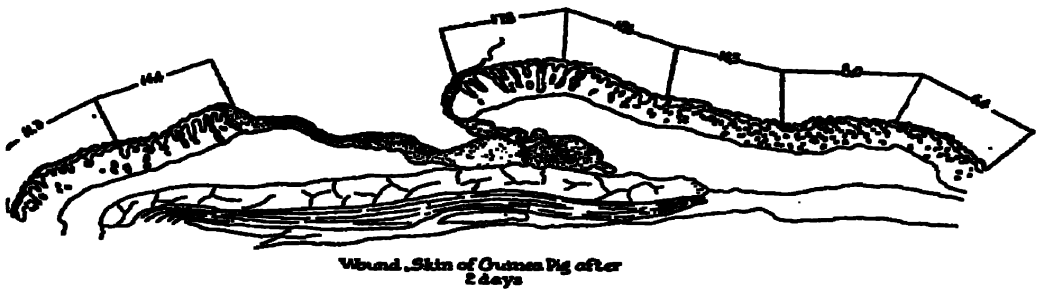

Fig. 4. Projection drawing of a thin cross-section of a 2 day old wound in the skin of the abdomen of a guinea pig. The average number of mitoses in 10 serial sections of the indicated areas of the epithelium are given in the figures above this layer. The exudate is shown in stipple. The whole skin at one side has move up over the wound.

animal is allowed to lie relaxed on his back it is circular. When the animal awakens and squats the longitudinal axis shortens and the transverse becomes the long axis of the wound. On the back the wound may gap to an irregular circular outline. The irregularities are the result of irregularities in the attachment of the skin to the deep fascia.

Changes in the Wound during Healing. After 6 to 24 hours these edges become fixed however and begin to move at first slowly and then more rapidly toward each other. In the wound on the abdomen and back this movement of the skin inward and shrinkage of the edge continues often until closure is practically effected, Fig. 3.

The layer of tissue which moves over the wounds includes the epidermis, dermis and a few attached fibres of the superficial fascia, Figs. 4 and 5. This forward movement of the skin layer leaves an open space between the skin above and deep

fascia below. This space is lined by fibres and cells of the superficial and deep fasciae. When this movement commences the connective tissue edges of the wound are oedematous, Fig. 4. They contain a scattering of leucocytes. No growth activity is seen in any of the connective tissue cells except in places where the deep muscle layers have been accidentally injured. Here

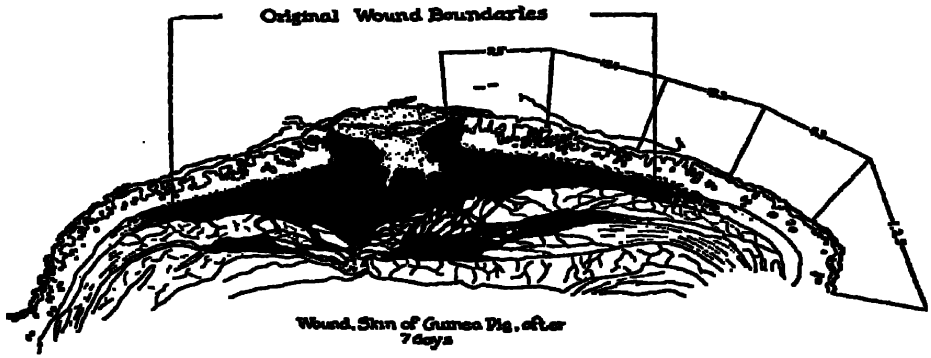

Fig. 5. Projection drawing of a thin stained cross-section of a 7 day old wound in the skin of the abdomen of a guinea pig. The average number of mitoses of the indicated areas of the epithelial layer are given in figures above this layer. The exudate is shown in stipple. The area of dense connective growth in the wound is in dead black. The areas of moderate growth are indicated by dashes. There is an area of proliferating connective tissue cells beneath the hyperplastic epithelial layer.

one may find a more active inflammatory reaction, a proliferation of fibroblasts in the tissue and an invasion of adjacent parts of the exudate by these cells.

The epithelial cells on the other hand are already active. This layer has already noticeably thickened not only at the wound boundary but far behind this point. This proliferation is greatest at or near the edge of the wound. It gradually decreases back from this point as is indicated in Figs. 4 and 5.

As the skin edges move forward the connective tissue cells begin to proliferate and to migrate into the exudate from the bottom and sides of the wound. They fill and close the gap between the deep fascia and skin above. This movement of the skin continues to the limits of its elasticity or until it becomes fixed by the connective tissue overgrowth. An expanding mass of granulations fills the intervening gap, Fig. 5. Later

an epithelial membrane stretches out over the granulations to complete the closure.

In the wounds of the skin of the back of the neck and ears the picture is identical except that the opening left to be closed by granulations is much greater. After a meager primary shrinking of the edges there is a period of rest when granulations spring up from the bottom of the wound and an epithelial membrane stretches out over them to effect a closure. In the cleaner wounds this movement of the epithelial cells takes place early and prevents any very great activity in the connective tissue cells. In most of the wounds the granulations grow sufficiently to fill the normal gap between the deep fascia and epidermis. In one wound the epithelial movement was more rapid so that the level of the freshly healed wound surface was below that of the surrounding skin.

While such a forward movement of the whole skin over the wound may be the result of a shrinkage of the edges of the wound, the evidence gleaned from the cultures and studies of similar wounds on the back of my hand indicate that it is the result of a force acting between the skin edge and the center of the wound. The gross movement of the skin described in the cultures above is the direct result of a forward migration of the epithelial cells. Many of the wounds on the back of my hand have closed so rapidly that they have shown definite radial wrinkles. The shrinkage of the edge is evidently either a secondary phenomenon which may or may not keep pace with the forward movement or it is a part of the same mechanism of migration.

The picture in these wounds is identical therefore to that seen in cultures excepting that the cells failed to grow in the plasma cultures while the movement in the wounds is associated with an active proliferation of cells. Where the edges of the wounds are mobile they move in mass towards the center of the wound. Where they are fixed, an epithelial membrane stretches out from them.

The Behavior of Various Types of Cells in these Wounds and the Sites of Greatest Proliferation. As stated above cellular growth in the cultures depends not only on food, oxygen and a

proper temperature but also on a crowding of the cells together in a stagnant environment. In these wounds cellular proliferation commences earliest in the epithelial layer. This layer has no intercellular capillary net. The cells in it are crowded together. This layer dominates not only at the onset but continues to dominate throughout the healing process. In none of these wounds have I found any evidence that this growth of cells follows any laws of purpose for healing. The cells in the epithelial layer not only grow and move out over the wound but they proliferate in situ for a long distance behind the wound's boundaries. The sites of proliferation in the epithelial layer correspond exactly to areas of the skin suffering from inflammatory congestion. The amount of this proliferation is proportional to the amount of congestion of blood in the areas below it. The migration and growth of these cells at the edge of the wounds are also from the less to the greater stagnant areas within the wound. Again, this proliferation is seen not only in the covering layer of epithelium but also in the glands and hair follicles. Hypertrichosis to a greater or less degree is practically universal about these healing wounds.

The earliest growth of connective tissue cells is seen in injured areas of muscle. Later the cells in the deep fascia, in the superficial fascia and the true skin proliferate in situ. Capillary sprouts and these proliferating cells then move into the exudate. The greatest growth is seen in these cells which invade the edge of the stagnant wound area. In this area they grow as a densely cellular expanding mass of tissue. The endothelial cells of the capillary sprouts also grow laterally as well as forward to form sinusoids, which are larger than the capillaries and vessels at the edge of the wound which supply blood to them.

Beneath the overlying skin this excessive growth of connective tissue continues only until the gap is filled. It then suffers atrophy and shrinkage. Through the contraction of this mass the edges of the superficial fascia are pulled together.

In the middle of the wound this growth of connective tissue cells reaches its greatest dimensions. Here this growth continues always until the wound is covered by the epithelial cells.

This is not true of the epithelial cells. The cells in this layer continue to grow for a long time afterward and apparently at the expense of the granulation tissue. In most of the wounds the epithelial cells stretch out as a straight layer over the granulations. In a few of the wounds, however, one may see irregular overgrowth from these epithelial membranes. These appear as papillary downgrowths into the underlying connective tissue. At no time have I found evidence that these downgrowths are other than abnormal. There is no evidence that they are to be later changed into hair follicles or glands.

This growth in the epithelial layer continues until the granulations have suffered their extreme atrophy. When the epithelial membrane first stretches over the granulations it is a double or single layer of cells. This subsequent growth leads to rapid increase in cells within it until it is often as thick as the lateral hypertrophic parts of the skin. Finally when the scar has formed and the blood vessels have either shrunk to delicate capillaries or disappeared, growth ceases also in this layer. The outer cells of this layer then become keratinized.

During the migration of the epithelial cells over the wound the original horny layer separates from the lower active cells of the epidermis. This horny layer need not be cast off at once. It often also migrates into the exudate for a considerable distance. It apparently never becomes attached again to the underlying cells of the epidermis. A new horny layer is later always formed from the outer layers of cell of the new epidermis.

The Rôle of the Exudate. Many textbooks of pathology state that the exudate is removed by leucocytes. In the cultures described above it has been noted that the epithelial cells may dissolve these clots. Polymorphonuclear leucocytes are seen in the areas of newly formed connective tissue at the edge of the wound and in the exudate until closure is effected. They then disappear apparently by way of the blood vessels. With the narrowing of the blood vessels and the increase in rate in the circulation these cells are apparently attracted out of the tissues.

In very few of the wounds studied has there been any evidence that these cells lead to any extensive dissolution of the

clot. They migrate into the exudate along narrow channels which they apparently dissolve ahead of themselves. Only when they are in very large numbers, however, does this dissolution become generalized.

Carrel [6] in a recent paper has tried to prove that they are the active stimulant for growth in these wounds. I find no evidence for this deduction. In fact they inhibit growth of the connective tissue cells in the cultures when in larger numbers. I have seen the most active growth always in wounds containing only a few of these cells. As I have pointed out in previous papers [7-11] the stimulus for a growth of cells in the cultures other than food, oxygen and a proper temperature, is stagnation. The whole of the metabolic reactions of the cells are induced and controlled by a certain substance or substances liberated by them through the oxidation of other substances within them. These products of the oxidation reaction act in proportion to their concentration. The body cells have no means to retain these products within themselves in an environment where they may readily diffuse or be washed away. In order for these cells to digest their food they must be crowded together in a stagnant environment so these products of their oxidative reaction can accumulate about them.

In most of these wounds, it is interesting to note that the connective tissue cells do not become dispersed in the exudate as in the cultures. The connective tissue grows in the wound as an expanding mass of cells and blood vessels. At an earlier time I had believed that the plasma clot supplies no food to the connective cells migrating and growing in the cultures. Later studies have shown, however, that this is probably not true. These cells except where they are very closely packed together cannot destroy the fibrin, but they do take fats, serum proteins, and evidently other substances from the clots. Their dispersion is probably largely due to their being attracted to these food substances in the medium. In the wound, conditions are different. Nutrient substances are probably largely derived from the dilated blood vessels within the granulation tissue. As I pointed out in previous articles the migrating and growing cells in the cultures may also draw nutrient substances

from other less favorably located cells in the center of the fragment. In a part of the cultures of fragments of the skin and glands of older embryos one sees a most active growth of mesenchyme cells. In many such fragments the epithelial cells gain the upper hand. They stretch out as a membrane, dissolve the clot and destroy the mesenchyme cells. In other cultures the mesenchyme gains control. These latter cells then grow at the expense of epithelial cells in the fragment. The degenerating epithelial cells supply the nutrition for the abundant growth of these cells observed in such cultures. These growing mesenchyme cells obtaining their nutrition from within the fragment then also grow as an expanding mass which dissolves and removes the clot ahead of it. Such an expansile growth continues, however, only until the epithelial cells are reduced to a granular mass. The mesenchyme cells in the mass then either degenerate or begin to disperse into the clot.

In the wounds one sees no such extensive dissolution of the clot about the epithelial layer as in the cultures. The epithelial cells generally move out over the surface of the granulation tissue. Where they move out into the exudate, they destroy it ahead and over them but not so much beneath them. Infected parts of the exudate are apparently either pushed outwards by the growing fixed tissue cells or dissolved by a massive accumulation of polymorphonuclear leucocytes within them. The cleaner parts of the exudate disappear on the other hand with the growth of the fixed tissue cells. They are apparently eaten up so to speak, by these growing cells.

Summary and Discussion

Since the work of Cohnheim the attention of students of wound healing has been largely centered about the behavior of the leucoyctes and the other wandering cells. With the advent of the tissue culture it has been possible to study the behavior of the fixed tissue cells and to analyze the general conditions which regulate their growth. Through a comparison of the behavior of these cells in the cultures and in the wounds it has been possible to explain certain variations of the healing proc-

ess in wounds, the importance for healing of the dominance of one tissue over another, how one tissue may thus gain control, and how injury induces growth in these cells.

While it has been shown that the leucocytes play an important rôle in removing infected areas of the exudate, bacteria, necrotic tissue and other irritating substance there is little evidence that they are of any importance, as Carrel contends, in otherwise stimulating and controlling the growth of the fixed tissue cells. An active growth of epithelial cells is seen in the areas behind the edges of the wound. These areas are frequently largely free of leucocytes. A careful study of the above wounds shows that the most active growth of cells is in the stagnant area of the wound. Outside this area the cellular epithelial layer and a narrow zone of the less cellular connective tissue undergoes proliferation. This proliferation corresponds exactly to the area of inflammation or the area suffering congestion or slowing of the circulation.

Several years ago Morgan [12] had shown that the growth in regenerating tissue is not due alone to food and oxygen. It is the result of other conditions. Morgan found that the legs of starving salamanders regenerate as rapidly as those of well fed ones. The difference between these animals is that the starving ones suffer an excessive atrophy of all of their organs. The regenerating tissue had taken its food supply evidently from the differentiated cells of the animals. By these experiments Morgan had not only shown the necessity of special conditions or stimuli other than food and oxygen for the cells to grow but he had also shown that the cells stimulated to grow may draw food substances directly from other cells less favorably located in the body.

Using the tissue culture I attempted to ascertain the significance of these facts and the general nature of the conditions or stimuli other than food and oxygen necessary for the growth of these body cells. In cultures of embryonal cells supplied with ample nutrition and oxygen for a considerable growth of cells I have found that these cells are unable to grow unless they are crowded together so that a certain product or products of their normal oxidation cannot escape from them. The growth of the

cell can be stopped by washing them with serum or by placing them in a relatively large amount of medium which adsorbs or keeps by other indirect means the concentration of this primary oxidation product or products below a certain amount. The energy utilized by the cells for drawing food to them, digesting and synthesizing it, for their migration and function is directly proportional to the concentration of this primary oxidation product or products within and about them. The concentration of this substance is directly proportional to the number of cells crowded in a given area and indirectly proportional to the stagnation of this area. In the body its formation is either prevented or it is removed by the circulating blood.[7, 8, 9, 10, 11] I have called this substance or substances the *archusia*, the driving substance of the cell.

In these previous papers I have shown that the hypertrophy of certain tissues, the growth of cells in the bed of the nail and in cancer is induced and controlled by the same conditions. It can be related to the densely cellular character of the tissue, a relatively poor circulation, or both.[8] In the wounds studied above the growth is not one of immediate purpose for healing. It is generally excessive and beyond the bounds of the wound. This growth as in cancer and other growing tissues corresponds to the areas suffering a reduction in circulation. The reduction in the circulation is the direct result of the destruction of blood vessels and the inflammatory reaction. The amount of growth is always proportional to the congestion and stagnation.

In the clean wounds of the skin of adults the dominating tissue is the epithelial layer. Growth is first seen in this layer. This layer may also move forward at an early time into the wound dragging the skin edges with it or it may stretch out as a membrane over the surface of the wound holding in check the growth of the granulation tissue. The granulation tissue grows in excess only in those wounds where the epithelial cells are injured by infection or otherwise. In the eventual healing of these infected granulating wounds the granulation tissue suffers atrophy and hyalinization. These later changes take place with the growth of the epithelial cells. These latter cells continue to grow until the hyaline scar is formed.

In the experiments with the cultures it has been shown that a dominating tissue may not only inhibit the growth of other cells but that these dominating cells may also destroy and use as food the protoplasm of these other cells. Contraction in skin wounds has been shown to be the result of the activity of the epithelial layer. In clean wounds where the skin is not attached to deeper structures the more active epithelial layer drags the edges of the wound together. In other areas where the skin is attached to deeper structures the epithelial cells stretch out in a membrane over a growing granulation tissue. These cells in their further growth destroy in part at least the granulation tissue and effect a secondary contraction of the wound. In the same manner the epithelial layer must destroy and grow at the expense of the granulation tissue at the time of the eventual healing of the infected granulating wound.

Morgan, as stated above, had shown that cells stimulated to grow may take substances necessary for their growth from less active cells of the organism. In the cultures I [5] have noted that mesenchyme cells of the embryo may grow at the expense of epithelial cells. Recently Barta [13] working in my laboratory has confirmed experiments mentioned above which illustrate the reverse dominance of the epithelial over the connective tissue cells in adult tissues. He stimulated the activity of the cells in fragments of the ureter of half grown rats by adding embryonic extract to the medium. In all but a very few cultures the epithelial cells grew at the expense of the muscle and the adjacent connective tissue cells. The muscularis and the submucosa of these fragments suffered a true hyalinization not unlike that seen in the granulation tissues of the wound as it becomes transformed into the scar.

These experiments have also shown that this dominance of the epithelial layer over the connective tissue in the adult is not related to any age change or any other peculiarity of the cell itself excepting those peculiarities which hold the epithelial cells in layers free from blood vessels and allow the connective cells to scatter in a vascular tissue. It is related directly to the cell density of the different tissues and the stagnation of their immediate environment. Throughout the work with the tissue

culture I have seen groups of cells prey as readily on other groups of their own kind as on cells of other kinds.

By these observations it has been possible, therefore, not only to become acquainted with certain parts of the mechanism of healing in skin wounds which allow a correlation between the various types of healing but also to give further confirmation that *cell crowding* and *stagnation* are the fundamental factors which regulate the normal balance between tissues, local hyperplasias and hypertrophies, atrophies, hyalinization, development, wound healing and cancer. These differences in the end result are due merely to quantitative differences in these fundamental factors. In the beginning of the life of the animal it has no circulation. It is with the gradual separation of cells and development of blood vessels that growth becomes irregular in the embryo, slows and ceases in adult life. In previous articles I have shown that the growth of the cells in the bed of the nail is related to a peculiar reduction in the circulation and a partial cutting off of the cells from the immediate effects of the circulation. Throughout the area of the lunula the epithelial layer is not attached to deeper structures.[14] The circulation under this part is reduced to a single layer of large dilated capillaries or sinusoids. These vessels are filled and emptied by vessels of smaller calibre. The circulation in these sinusoids must be sluggish. A similar type of blood vessel is found in the bone marrow, the developing liver and in granulation tissue. Hypertrophy of the heart follows a primary dilatation of the heart and a compression of its blood vessels. The fingers club in congenital heart and in many lung diseases.

Cancer, as stated above and in previous articles is the result of a reduction in the circulation of a cellular tissue or to the crowding of cells together and a corresponding reduction in the circulation of the newly formed mass. Viscid drops of coal tar are able to induce cancer in that they are able to attract fixed tissue cells to them. The cells attracted are not only the epithelial and connective tissue cells but the endothelial cells of the capillaries and the smaller blood vessels. These drops of coal tar are thus able not only to build a cellular tissue about themselves but one having a reduced circulation. The blood

vessels about these drops are destroyed through the attraction of their endothelial cells away from them. These drops of coal tar do not show any greater affinity for one type than another of these fixed tissue cells. The greater number of connective tissue or the epithelial cells in such masses, as the case may be, is related to the position of the drop of tar. If the epithelial cells predominate in the tissue where the tar is placed, they collect in greater numbers about it. The number of cells collecting about any drop of coal tar is also proportional to the number of cells originally present in the tissue. In the sparsely cellular subcutaneous tissue of adults only a few cells collect about the tar. The mass of these cells is never great enough to induce growth. Many of its blood vessels have been destroyed, however, so it soon becomes transformed into a hyaline scar. In the cellular mesenchyme of embryos sarcomatous growths develop readily. Drops of tar placed against the epithelial layer of adult skin collect dense masses of epithelial cells about them. When these masses become large enough their cells either degenerate or grow and destroy neighboring normal tissue.

So in the same manner evidence has been found to show that the growth in the wound is also the result of a similar stagnation. The question arises why does the growth cease in the wound and continue in the cancer. The above observations answer this question by showing that the stagnation produced in the cancer is the result of the building of a new tissue having an overabundance of certain cells and a greatly reduced blood supply. These larger dense masses of cells are not only able to invade and destroy neighboring tissues and blood vessels but they may prey directly on their own blood vessels and stroma. Ball has pointed out the peculiar shrinking of vessels in many cancers.[15] In other cancers one sees many large dilated vessels. In the cultures a small fragment of cancer cells will eat and thus destroy normal endothelial cells as well as other cells. Recently Fischer [16] has shown that the cells of a chicken sarcoma can be kept growing in a tissue culture for a very long time by feeding them normal muscle of a chicken. As it is interesting to note, however, these same cancer cells under the proper conditions may also stimulate normal cells to grow.

Cancer cells undergoing degeneration in the tissue culture liberate a fluid which is a most active stimulus for normal cells. Salt solution extracts of cancer also stimulate cells to grow when added to the medium of a tissue culture or injected into the body. It is not surprising therefore that one may see an excessive growth of stroma and large dilated blood vessels in certain parts of malignant tumors. These vessels and tissues, however, are always eventually destroyed. Cancerous tissues continue to grow because a sufficient mass of any cells, once built, continues to destroy other cells and its own circulation. Such a destruction of blood vessels leads to a degeneration of a part of the mass as it also forms conditions suitable for the active growth of other parts.

In a recent unpublished study of the lymph glands from 60 cases of sarcoma and carcinoma I found that the glands, which receive fluid from the malignant tissue, had suffered hyperplasia. This hyperplasia was often excessive. The endothelial cells of the sinuses as well as the follicles had grown excessively. The sinuses were choked with these cells. This growth had continued until the cancer cells reached the gland. The glandular tissue was then eaten up by the growing cancer cells (see also Ewing,[15] page 78).

I have been unable to find that the isolated cancer cell is different from a similarly isolated normal cell which has remained for a short time in a fragment placed in the stagnant culture. The growth in cancer is not the result of a primary change in the cell but the direct result of a crowding of the cells together and a proper reduction of their blood supply. These crowded masses transform more energy than neighboring tissues and their stroma. Growth ceases in most wounds because the blood vessels grow with the fibroblasts. In the ordinary wound the blood supply is never reduced nor the cellular constituents increased in such proportions that one may prey on the other without an eventual loss to itself. As is evident, however, only a slight deviation in this balance may lead to cancer. It is known that cancer may follow simple injury and that it frequently develops in skin suffering atrophy from a decrease in blood supply, and in scars.

In a further study of the effect of reducing the blood supply it was of interest to note that this change alone will not cause cancer in the normal adult skin. The epithelial cells of the adult skin are not in numbers sufficient to grow independently when their blood supply alone is reduced. The cells will not grow from fragments of this tissue placed in the cultures. I [17] have reduced the blood supply to the normal skin by exerting continuous pressure upon it. In every case atrophy and simple ulceration have been the results. Many of these ulcers have persisted for months but none of them has ever become cancerous. If, on the other hand, I thickened these epithelial layers by first injecting a salt solution extract of an actively growing tissue into the skin, a few cancers have followed the application of the continuous pressure.

In a more cellular tissue a reduction in blood supply is apparently ample. I destroyed the blood supply of fragments of mesenchyme of rat embryos. A few of these have developed into the most malignant sarcomata when transplanted into the subcutaneous tissue of a young rat. These vessels are readily destroyed by treating the fragments for a time with an extract of an actively growing tissue or by placing the fragments in a drop of plasma. Under these conditions the endothelial cells migrate apart and to the surface of the fragment. The vessels thus disappear. The same endothelial cells of fresh untreated fragments retain their arrangement when transplanted into the animal. These vessels connect with those of the host. The circulation in these fragments is thus normally reëstablished. The fragments grow to form benign embryomata. In the same manner a cellular pigmented mole becomes malignant often after slight injury. The mere destruction of its blood vessels is apparently ample to excite a malignant proliferation in it.

As is evident from the above observations, such disproportions between connective tissue cells and their blood must be easily produced in the cellular mesenchyme and connective tissue of embryos and young adults and difficultly produced in these cells in the adult. In later life it is not surprising, therefore, that sarcomata are not common excepting as they may arise from the cellular layers of the periosteum and endosteum

while carcinoma is the prevalent disease of other regions of the body at this time.

Thiersch and Remak had noted an antagonism between epithelial and connective tissue cells during development and in later life. They recognized that cancer may be nothing more than a break in the balance between these tissues. What they lacked to correlate this idea with other natural phenomena was a knowledge of the conditions which regulate the transformation of energy in the cell and control this balance.

Previous work has shown that the energy for living things is derived directly or indirectly through the oxidation of organic substances. Schwann had noted the similarity of body cells to unicellular organisms and thought them capable of an independent existence. He thought they differed from the unicellular organisms in that they had learned to live together. Herbert Spencer had pointed out the condition of specialization in these cells: how one had become adapted for defense, another for digesting food, another for movement, etc. Later workers have shown even a greater dependence of any group of these cells on others for their metabolism. This is especially prominent in the studies of the glands of internal secretion.

While it is true that such specialization has made these cells wholly dependent on the colony or the whole for their existence, there is no reason to believe that this specialization is the primary cause of the colonial existence which they enjoy. It is more likely the result, as the careful studies of Driesch, Hertwig, Wilson and others [18] have shown. Dependence and specialization is the result of the overcrowding of a community and not the cause of the overcrowding.

It has been our good fortune to find that the primary factor which forces these cells to live together is that they have no means to retain and transform the energy liberated in their oxidation reaction when isolated in nature. To retain this energy they must be crowded together. They have either never developed structure suitable for properly forming or retaining their primary oxidative product or products, the *archusia*, or they have lost these structures. Whenever body cells are isolated they soon become powerless to perform any of their acts

(Burrows, 1913).[19] These primary oxidation products are essential for all other reactions of their metabolism.

In a study of these cells of man and higher animals I have found, therefore, a simplicity of structure which has allowed one to separate certain parts of the mechanism which produces growth and to find its energy is transmitted from the oxidation of certain food substances in the form of an extractible product or products, the *archusia*. This product acts according to its concentration.[20] The body cells become subjected to the whole only through the development of the circulation which either prevents the formation or continuously removes this product from about the cells. When these cells become sufficiently crowded together and their circulation properly reduced they may become independent growing systems which like a parasite are able to eat up or destroy the body. Thus it has been possible for the first time to give a plausible explanation of cancer and to define the stimulus for growth in wounds.

Conclusions

1. Clean wounds may heal either by an early extension outwards of an epithelial membrane and granulations from beneath or by a gross moving together of their edges and a later cementing of these edges to the deeper structures and to each other by granulation tissue.

2. In those parts of the body where the skin is firmly fixed to outside structures the former type of healing prevails. In other parts where the skin is freely mobile, the latter type is seen.

3. The stimulus for growth after injury is the destruction of blood vessels and the inflammatory slowing of the circulation.

4. The reaction of growth in the wound is not one of purpose for healing. The amount of cellular proliferation is proportional only to the crowding of the cells and the stagnation of their environment. The cells not only grow but also migrate actively into the areas having a poor circulation. It is this tendency for cells to migrate and grow in areas of reduced circulation or the stagnant areas of the open wound which leads to the closure and the overgrowths of the wound.

5. The closure is effected more rapidly at the surface because of the greater activity of the epithelial cells. This greater activity of these cells is related to their being crowded together in a layer free from an intercellular capillary net.

6. The late contraction of the wound which is of little importance in the simple wounds studied above but which becomes important in granulating wounds is due to the atrophy of the overgrowth of the granulations.

7. All evidence points to the fact that this late contraction is due to the activity of the epithelial cells which continue to grow to the time of the completion of the healing process and draw their nutrition directly from this mass of granulation tissue. Densely cellular masses of any tissue having a stagnant environment may take the protoplasm or essential parts of it from other less densely crowded cells.

8. The stimulus for growth in the wound is not different from that in cancer. Both are due to external conditions which produce stagnation sufficient to allow either the formation or the accumulation of the normal oxidation product or products of the cells about them. In the healing wound the primary changes are insufficient to prevent the return of a balance between cells and blood vessels. Cancer results when the primary changes are such that there is an excessive overproduction of one group of cells. This larger mass of cells can then prey on normal cells and destroy its own circulation about it. It thus not only leads to the destruction of large parts of itself but builds conditions suitable for the continuation of growth in other of its parts.

9. Cancer may be caused by any substance or conditions which lead to the proper primary change in the arrangement of cells and blood vessels in the organism.

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DESCRIPTION OF PLATE LXXVII

Fig. 6. A reconstructed drawing of a fragment of the skin of a 15-day old chick embryo in a layer of plasma swung on a cover glass which is not shown. (*a*) Is the epithelial membrane which has been stretched by a local contraction of the clot.

Fig. 7. A drawing of a paper-clay model of one side of a culture of frog skin. The membrane of epithelial cells is shown stretched between the edge of the plasma clot and the fragment. The nuclei of the epithelial cells are shown dark like in a stained preparation.

Fig. 8. (*a*) Camera lucida drawing of a buttonhole cut in a fragment of skin from an 18-day old chick embryo. The epithelial layer was cut away from one side of the wound. (*b*) The same wound 30 hours later. The sharply outlined border of the buttonhole is the part covered by the epithelial cells.

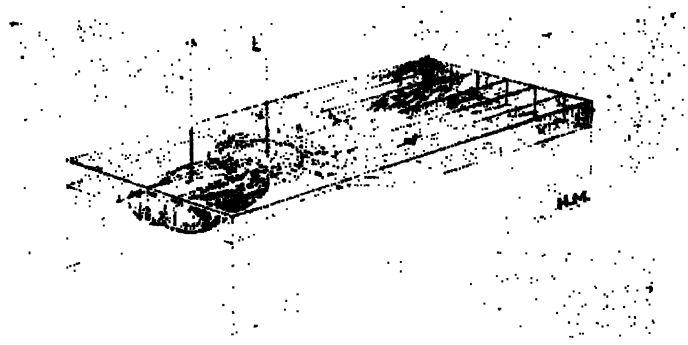

Fig. 6

Fig. 7

Fig. 8a

Fig. 8b

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115 (2638)

Studies to determine the biological significance of the vitamins.

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Earlier authors have fully appreciated that conditions other than food and oxygen are necessary for an active growth of cells in the body. This fact is well exemplified in the work of Morgan[1] on the regeneration of the legs of salamanders. Morgan noted that the legs of these animals regenerate as rapidly in starved as in well fed animals. I have sought these other conditions by means of the tissue culture, and find they are a *crowding of the cells* and *stagnation*. Single isolated cells or small groups of cells will not grow in a drop of plasma. For these cells to grow they must be crowded with other cells to form a compact mass of considerable size, and be placed in a small amount of stagnant medium so that the loss of soluble materials from the mass is reduced to a minimum. In the presence of oxygen the cells in such a mass begin to grow after a given latent period.[2]

In analyzing these factors of cell crowding, stagnation, oxygen and latent period more carefully, I have further found that they signify that growth depends on the accumulation of a certain concentration of an oxydative product of these cells. This product can be readily extracted with salt solution, and when added in a certain concentration to a drop of plasma it will stimulate growth

[1] Morgan, T. H., *Jour, Exp. Zool.*, 1906, iii, 457.

[2] Burrows, M. T., Trans. *Cong. Am. Phys. and Surgeons*, 1913, ix, 77.

in isolated cells placed in the mixture. In lower concentrations (S_2) it stimulates these cells to migrate and store proteins and fats. Only in certain high concentrations (S_3) can the cells digest these proteins and fats and grow. In all higher concentrations (S_4) it leads to the digestion of the protoplasm of the cells themselves. I have named this substance or substances the *archusia,* the driving substance of the cell.[3, 4] It corresponds to the heat in the steam engine. It cannot accumulate in any tissue having a rich and active blood supply unless the blood has become saturated with it from other sources.

Through the careful extraction of the various tissues of the body it has been found that the growth rate of these tissues corresponds to the amount of *archusia* contained within them. This concentration of the *archusia*, it being a normal product of the oxydative reaction of the cells, can be increased by crowding the cells and reducing the circulation to them, or it may be maintained from outside sources. Cancer I have shown to be nothing more than the result of a primary crowding of the cells together and a relative reduction in their blood supply. As the crowding and the stagnation reaches certain proportions, the mass acquires the property of independent growth. This mass continues to reproduce itself in that it preys upon and destroys surrounding tissues and blood vessels.[5]

The active growth in the bone marrow, nails, sex glands and in wounds is maintained by the same conditions of stagnation of circulation and cell crowding.[5] These factors are only less in degree than in cancer. In development the greatest growth is in early life. This growth wanes with the development of the blood vascular system and an active circulation. The embryonic tissue of the 5 day old chick-embryo differs from cancer only in that the number of cells per unit capillary area is greater in cancer or the blood supply is less. To transform an embryonic or adult fragment to cancer, it is necessary to stimulate a growth of cells outside the blood vessels (to increase the cells per unit capillary area), or reduce the number of capillaries in the fragment without disturbing the cells. Unchanged embryonic fragments when transplanted into a host form benign tumors; when

[3] Burrows, M. T., *South. Med. Jour.*, 1924, xvii, 233.
[4] Burrows, M. T., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 94.
[5] Burrows, M. T., *J. Med. Research*, 1924, xliv, 615.

the cells in them are increased proportionally over the blood vessels they become malignant tumors when transplanted to a host.[6]

By these observations and others on coal tar,[7, 8] etc., we have found the nature of the cancerous organization and the manner by which it is brought into existence. How the normal functioning organism is developed, and how it maintains its organization remained for solution. In comparing the amount of stagnation and cell crowding necessary for a growth of body cells, it was noticed that most tissues, even those of the fœtus and the young child, are too richly supplied with blood for growth to intervene, unless their blood be supplied with growth stimulus from other sources. We have sought a source for this stimulus in the glands of internal secretion and in the food. Johnston and I[8] have studied by a new method the action of the ovary in this capacity. We have found that the Allen, Doisy hormone stimulates an active digestion of fat and an active growth of the subcutaneous connective tissue cells. Other authors have noted its action on the uterus, breasts and other sexual organs.

Edwin Smith in his work on the production of tumors by the *B. tumefaciens* showed that this organism stimulates an active growth of cells in the plant. Recently Blumenthal, Auler and Meyer[9] have isolated a similar organism from human cancers, and reproduced cancers in plants and animals. The action of this organism on the animal cells is quite identical to the extracts of any actively growing tissue from the animal as noted above. It became of interest to see what might be the action of this organism when fed to animals. Jorstad and I have fed two day old cultures of this organism as well as similar cultures of the *B. campestris,* an organism which produces a primary stimulation and then a destruction of plant cells. Chamber[10] while working here in the laboratory showed that the *B. campestris* destroys the plant cells through its ability to break up starch. This ability to split starches does not develop early but late in the cultural life of the organism.

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[6] Burrows, M. T., *J. Mo. State Med. Assoc.*, 1923, xx, 145.

[7] Jorstad, L. H., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 67.

[8] Presented 1924, *Am. Soc. Exp. Path.*, Washington, D. C.

[9] Blumenthal, Auler and Meyer, *Zeitsch. fur Krebsforsch.*, 1924, xxi, 387.

[10] Article in Press.

For controls animals were fed:

Potato Starch	80 grams
Egg Albumin	50 "
McCollum Salt Mixture	10 "
Autolyzed Yeast (Vegex[11])	10 "
Butter	30 "

In the experiment 20 cc. of a 2 day old culture of the organism in a simple potato decoction were substitued for the yeast. In other experiments the butter was replaced by crisco to determine if the organism replaces vitamin B (yeast), vitamin A, or both.

In the accompanying curve 1, the results of one of these experiments are given. Both bacteria act in a diet with butter as active and normal growth stimuli. Growth fails when the butter is left out of the diet. These bacteria replace vitamin B, but contain no noticeable amount of vitamin A.[11]

As is noticed therefore, these organisms when placed outside the vessels or into the tissue, stimulate a densely cellular and non-vascular or cancerous organization. When transmitted inside the blood vessels where they act on the endothelial cells first they stimulate the growth of a normal vascular and functioning tissue.

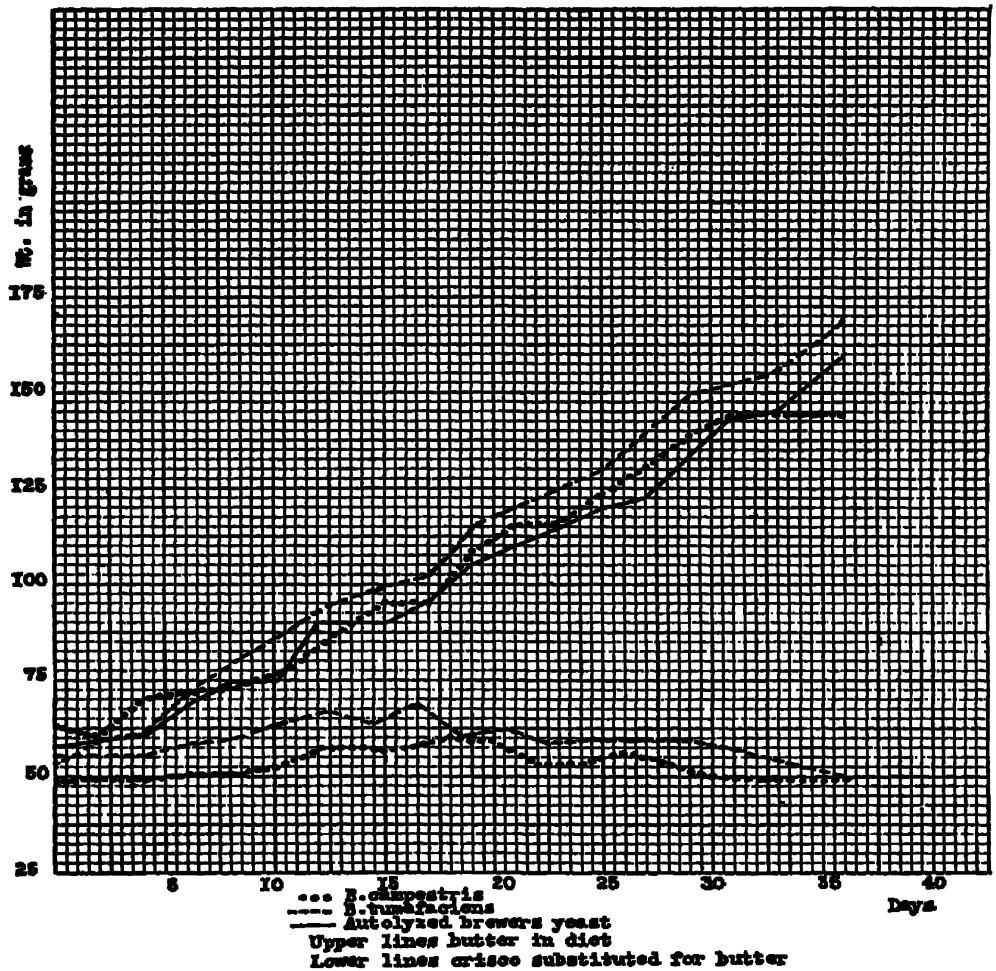
In other papers on muscular contraction,[12] I have shown that function is the result of a polarization of the cell. This polarization is induced by the growth of blood vessels and the rate of circulation. This increased blood supply inhibits growth and utilizes the same energy for function. It is possible, as the above experiments indicate, that the body survives in that it has certain internally secreting glands adapted to liberate stimuli and in preying on lower growing and non-functioning forms. If this be true the higher function in animals must be purely the result of an evolutionary development. These higher types have not only developed their vascular organization and function, but survive because they can prey for a part of their life's energy on lower non-functioning forms, and have glands which liberate *archusia* into their circulating medium.

By these observations it has been possible to throw light not only on the nature of these formative mechanism of development and the evolution of function in higher animals, but also on the biological significance of vitamins, and to give further proof that cancer is nothing more than the result of an abnormal arrange-

[11] Supplied by The Vitamin Food Co., Westfield, Mass.

[12] Burrows, M. T., *Am. J. Physiol.*, 1917-18, xlv, 556.

ment of cells. Normal development proceeds when a normal growth stimulus is transmitted through the blood stream where it acts to cause a primary development of blood vessels and secondary development of the tissues. Cancer develops when the same stimulus is placed outside the blood vessels or into the tissues so that the cells grow to form a densely cellular and non-vascular tissue. We have shown that coal tar acts in the same capacity, not by stimulating cells to proliferate and thus to form the non-vascular and cellular tissue, but by attracting cells from a wide



CURVE NO. 1. The three upper lines show the growth of rats fed on a diet containing butter and respectively *B. campestris*, *B. tumefaciens* and vegex. The two lower lines show the growth of rats on a diet containing *B. tumefaciens* and *B. compestris* respectively, but no butter.

area of the tissue and collecting them in dense masses about drops of it. Growth intervenes as these cellular masses become sufficiently large and stagnant.

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IS CANCER A TRUE DISEASE OR MERELY THE RESULT OF A CONDITION OF CHANGE IN THE GENERAL ORGANIZATION OF THE ORGANISM?

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IS CANCER A TRUE DISEASE OR MERELY THE RESULT OF A CONDITION OF CHANGE IN THE GENERAL ORGANIZATION OF THE ORGANISM?[1]

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THE problem that confronts the X-ray and radium therapist is not one of cancer prophylaxis, but the cure of the disease fully established. Many of the older writers had already concluded that cancer is not a disease in the sense that typhoid fever is a disease. It is not the result of a specific foreign parasite, but merely the result of some change, either in the cell or its environment. This idea is well exemplified in the work of Thiersch, Remak, Boll, Cohnheim, Ribbert, and others. Other authors in recent and older times have taken the other view. This other view has been recently revived by Edwin Smith, Ochsner and Nuzum. Smith based his conclusion on the identity of a cancer-producing organism in the crown gall of plants, the *B. tumefaciens*. Ochsner draws his conclusion from the fact that Nuzum has demonstrated bacteria in certain human cancers and has apparently excited the development o the disease by inoculating them into animals.

Against this parasitic view of cancer comes the interesting and recent work with coal tar, X-ray, radium, etc. While this work has shown that cancer may be induced by any one of a ber of conditions and substances, such as bacteria, coal tar, other lipoid solvents, X-r ty, radium, chronic inflammation, congen tal tumors and defects, etc., it has not s own that the cancers, once induced, are in any way concerned with any of these conditions or substances. It is too late for the Irishman to discard his pipe after his cancer has developed. Coal tar is essential in developing coal tar cancers, but the cancers, once induced, then proceed independently of the tar. Many cancers thus induced have been transplanted through many generations of animals. These cancers have grown undisturbed long after the coal tar had entirely disappeared.

The impelling forces of cancer, as seen in the light of these facts, are in the cell or the environment about the cell. The growth in cancer is not due to external agents, but to the relieving of the cell from the influences of the body so that it may grow and divide independently. Cancer will become known, therefore, when those general conditions, which regulate growth and function in body cells, become known.

It thus became of interest to study the general conditions regulating the growth of body cells as they are seen in the tissue culture and to deduce, if possible, the relation of these conditions to the general conditions peculiar for the growth of cells in the body. The theories deduced by the older writers, one may readily group into two classes: one of these, of which the Cohnheim theory is paramount, assumed the change to be primarily in the cell; the other view, of which Ribbert is one of the chief supporters, considered the change to be in the environment about the cell.

As Ribbert clearly points out, there is no evidence that the cells of the mature organism have lost their power to grow. The fact that they grow readily after injury rules out this possibility. Growth and function are sometimes determined largely by the environment about the cell. While Ribbert and the school which followed him failed to find the nature of the environment suitable for an active independent growth of cells and the impelling forces for growth in the wound, the exponents of the change being primarily in the cell lost ground continuously, as the various morphological peculiarities of the cancer cells were shown to be readily reproduced by producing the

[1]Read before the Radiological Society of North America, at Kansas City, December, 1924.

proper environment about normal cells. Among these peculiarities are the cell inclusions, the staining properties of the cells, the shape of the cells, their arrangement and peculiarities of division, and the formation of their chromosomes. Again the earlier writers, such as Driesch, Hofmeister, Sachs and DeBary, had already noted a definite relation between growth and the structure of the whole organism and not one related to the cell. Driesch's work has also shown that the growth of the cell in the normal organism may be influenced by mechanical as well as chemical conditions in its environment. He found that when the gastrula of the sea urchin is cut into two, the two halves do not grow at once, but only after each half has reformed into a perfect gastrula of one-half the size. This remolding is the result of a movement of the cells. Growth does not take place until this remolding is complete.

Bardeen and Morgan have shown that the growth of regenerating tissue does not depend on food and oxygen alone. Morgan finds the legs of salamanders regenerate as rapidly in starved as in well-fed animals. The difference between the two is that the starved animals suffer extreme emaciation from this growth, as well as from the lack of food.

The nature of these more subtle factors or stimuli regulating and controlling growth has not been found. In my early studies with the tissue culture, I undertook this problem. In these studies (1) (2) I noted that body cells will not grow under all conditions in a culture, when blood plasma is used as a medium. For these cells to grow is necessary that they be crowded together a stagnant area of medium. Single cells ill not grow. Growth intervenes only bout densely cellular and compact fragments placed in a small amount of medium. Growth may be inhibited by teasing these fragments apart and by placing them in a large amount of medium, which can more easily absorb and remove products formed by them.

Under these conditions of stagnation and crowding, it was further noticed that growth does not commence at once, but always after a latent period. This latent period is much shorter about fragments of an actively growing tissue, such as tissues of embryos, cancer and growing granulation tissue, than about normal non-growing adult tissue. As the growth rate of any organ progressively decreases from early to late life, so fragments of this organ placed in a culture show a progressively longer and longer latent period, before any activity is seen in their cells. The cells from a millimeter thick fragment of a five-day-old chick-embryo will begin to migrate as early as one to two hours after the cultures are prepared. Growth is well established in these cells twelve to twenty-four hours later. No migration of cells is seen from similar fragments of a ten-day-old chick-embryo fragment before six to eleven hours, and growth rarely intervenes before twenty-four to forty-eight hours. About fragments of a fifteen-day-old chick-embryo heart, migration begins after twelve to twenty-four hours (3). Growth may intervene after forty-eight to ninety-six hours, but it may fail entirely unless these older fragments are transplanted to new drops of plasma every twenty-four hours for three to several times.

This necessity of stagnation and cell-crowding for growth indicates that this process must depend on the accumulation of some products in the tissue fragments. The latent period shows again that this must be true and that this product or products must be formed by the cells.

To prove the existence of such a substance, I extracted, immediately after they were removed from the body, many fragments of normal non-growing adult tissue, the slower growing tissues of older embryos and the actively growing tissues of young embryos and cancer. Small fragments of these same tissues were also placed in stagnant hanging drops of plasma, where they were amply supplied with oxygen, but otherwise freed from any effect of the cir-

culating blood of the body. After several hours or days these fragments were removed and extracted in the same manner as the fresh tissues had been extracted. The extracts of all of these tissues were made by grinding in a mortar, equal weights of tissue with an equal weight of isotonic salt solution. The clear supernatant fluid was then added in equal parts to plasma and used for medium. Carefully washed isolated embryonic cells were used for testing. These isolated cells will not grow in pure plasma or plasma diluted with isotonic salt solution. In the presence of the extracts of growing tissues from the body, such as the tissues of young embryos, cancer and granulation tissue, these isolated cells showed migration and, in many instances, active growth. No such effects were obtained from the extracts of the tissue of older embryos and adults, when the extraction was made as soon as the tissue was removed from the body. On the other hand, if these same fragments of adult tissue are left in a stagnant drop of plasma, which is supplied with oxygen, they develop this substance in the course of one to six days, depending on their age.

In a further analysis, I attempted to see, then, how this substance is formed by the cells in the stagnant cultures, whether the actual quantity of it present in the tissues varies the reaction of the cells and is important in determining the growth reaction and (3) whether it might be washed away from actively growing cells by the circulating blood in the body. It has been seen that many fragments do not contain the stimulus when removed from the body. They develop it after they are taken away from their normal circulation and placed in the stagnant culture in contact with the same blood plasma which had previously bathed them in the organism.

In studying the formation of this substance in fragments of tissue in the culture, it was noted that it failed to accumulate, except in the fragments well supplied with oxygen, and the amount formed is directly proportional to the number of cells per unit area in the fragment, and the amount of oxygen consumed. In the fragments of older tissues, which contained no noticeable amount when extracted in the fresh, it took more oxygen to accumulate a measurable amount of this stimulus than in the younger fragments which already contained a limited amount (4).

From these observations, it became evident that this stimulus is a product of the cell's oxydations. In further experiments, I attempted to see if oxygen is necessary for the other reaction of the growth of these cells. In these experiments it was found that growth will proceed in the absence of oxygen as long as the stimulus is present (4). Oxygen, as it became evident, acts only in producing the stimulus. This led me, therefore, to look at this stimulating substance as something comparable to the heat of the steam engine and to name it the *archusia* or driving substance of the cell. It was evidently the accelerator of all reaction of the cell (5).

Further quantitative studies of extracts of young embryonic tissue and cancers, which contained large quantities of the *archusia* (S) showed that low dilutions (S^1) of it have no effect on isolated cells. In slightly greater concentrations (S_2) it acts to stimulate the cells to migrate into fixed proteins and fats and to take up mobile particles of protein and fat from the medium, and store them. In still higher concentrations (S_3) the cell digests the protein and fats and grows and divides. In all higher concentrations (S_4) the cells themselves are digested (6).

In many cultures, I forced a stream of serum, 1 part, and isotonic NaCl solution, 1 part, over and through the medium about actively growing cells. The diluted serum was passed along the fibers of a cotton wick, laid in the medium. When the serum is passing, growth is inhibited. When it is stopped, growth intervenes (7).

The effects of such an accumulation of products of the cell and its necessity for growth are easily shown in cultures where two fragments of any tissue are placed close

together. In the interval between two such fragments, substances coming from both fragments accumulate in greater concentration between the fragments than about other parts of the periphery of either fragment. Figure 1 is a photograph of two ¾ mm.

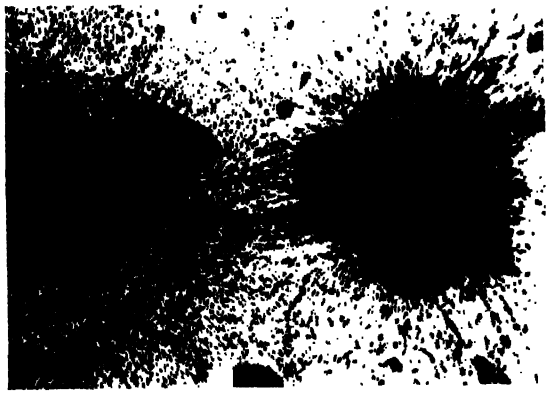

Fig. 1. A photograph of a simple hanging drop culture of two fragments of the eyeball of an eleven-day-old chick-embryo, showing greater growth at the interval between the fragments than elsewhere.

thick fragments of the eyeball of a chick-embryo placed ¾ mm. from each other. Note the active and greater growth of cells in the interval between the fragments.

It became evident in the light of these facts that a growth of cells in the body may be due to two factors; the passage to them of the growth-stimulating substance from other sources, or to a crowding of the cells together and a reduction in the blood supply to the mass. By the latter mechanical changes they were able to either induce the formation or accumulate this growth-stimulating substance in and about themselves.

It thus became of interest to see if all actively growing tissues of the body had this particular organization. It is a well known fact that the nails, hair and bone marrow have an independent growth in the organism. The most active normal growth is in the earliest periods of embryonic life. This active independent growth wanes as the blood vessels develop and the cells become separated. During the very active growth of the liver its circulation is through large sinusoids and is sluggish. I have found that the circulation at the outer end of the nail bed is reduced to a single layer of large sinusoids, which are filled by vessels of much smaller caliber. The bone marrow circulation is also sinusoidal and sluggish. Cancerous tissue is characterized by being densely cellular. Boll, many years ago, noted the peculiar, irregular, distorted and partly occluded blood vessels in many cancers. The capillaries are few in proportion to the cells in all cancers. In other cancers the blood vessels are large and the circulation is sluggish. In all cancers blood vessels are being destroyed at all times in the more crowded regions. In the wound, as I noted in a recent paper, the growth of cells is not related to the defect, but to the areas which are suffering from a reduction in their circulation either through a previous destruction of their blood vessels or an inflammatory slowing of their circulation (8).

In the studies on wound healing (8), I also noted that any dense stagnant mass of cells in a tissue not only grow, but they prey on neighboring tissues and blood vessels. They reduce these less crowded neighboring tissues to a hyaline mass. Such a dense mass of cells, as is found in cancer, may thus continue to reproduce itself and grow indefinitely. It destroys the normal blood supply and tissues ahead of it and grows continuously to form a dense mass of cells which degenerates in its central portions.

In the experiments with human and animal cancers there has been no evidence to show that the cancer cells are different in their requirements for growth than the normal cells. Single cancer cells cannot grow in plasma. The growth of these cells demands the same crowding as the growth of normal cells. The cancerous tissues differ from the normal cells in that the fresh extractives are somewhat more toxic than those of equally active growing embryonic tissues. In the cultures normal cells degenerate after an active period of growth. This degeneration comes on more quick-

ly in cultures of cancer. This is in proportion to the original greater crowding of the cells in the cancer. Whether it may be related also to other factors in the environment of the adult where cancer

Fig. 2. A photograph of a rat having a sarcoma produced by altering the arrangement of cells in a fragment of normal embryonic tissue transplanted beneath its skin.

exists, I do not know. It is possible that both these factors play a rôle.

From these observations it became evident therefore that cancer may be nothing more than the result of any condition or substance which may primarily reduce the circulation to a cellular tissue or build a local dense mass of cells, which has a lowered circulation. To prove this idea, it became of interest to see if cancer may be produced by such procedures. In the tissue culture the cells of cellular tissue fragment disperse into the medium. Previously formed structures may be thus rent asunder. Blood vessels are destroyed by their endothelial cells moving apart. The *archusia* extracted from actively growing tissues also stimulates the cells to grow in these fragments. These cells do not grow to form normal structure—they grow to form dense masses. Structures such as the blood vessels, epithelial tubes, etc., are lost in this proliferation. Fragments of fifteen- and sixteen-day embryos were treated, therefore, for twenty minutes with a Berkefeldt filtrate of an actively growing sarcoma and injected with the filtrate under the skin of adult rats. In 91 per cent of the animals the tissues of the fifteen-day-old rat embryos failed to grow to any extent. The

Fig. 3. A photograph of a section of a carcinomatous ulcer developing after four months of continuous low pressure on an area of skin of an adult rat, which had been injected at the beginning of the experiment with 1 c.c. of a Berkefeldt filtrate of a Jensen rat sarcoma.

blood vessels had been completely destroyed in these fragments. In 9 per cent, the growth was greatly stimulated and a sarcoma developed in one of these. This was a very malignant tumor. It was transplanted through nine generations of rats and proved fatal in over 95 per cent of the cases. These fragments had suffered only a decrease in their blood vessels. Sixteen per cent of the tissues of the sixteen-day-old rat embryos were stimulated and one sarcoma of the same kind as the former developed (Fig. 2). In the other rats the tissues of the sixteen-day-old embryos failed to grow in the presence of this high concentration of *archusia*. In other experiments, I tried the effect of a continuous reduction in blood supply to the normal skin by applying a continuous pressure. Atrophy and simple ulceration alone resulted. Fifty simple ulcers thus produced remained as long as six months to a year and a half, without showing any malignancy. In a third group of animals, I injected extracts of thirteen-day rat embryos or a Berkefeldt filtrate of a Jensen sarcoma into the skin

before applying the pressure. In these cases the cells of the epidermis and gland suffered hyperplasia. The atrophy and ulceration, which followed the action of the pressure in the previous cases, were either greatly delayed or carcinomata developed (9) (Fig. 3).

Having thus established by direct experiments that cancer may be the result of these simple changes in organization, it became of interest to see how coal tar, other lipoid solvents, bacteria and other agents produce this disease.

THE ACTION OF COAL TAR AND OTHER LIPOID SOLVENTS

In a study of cellular migration, it was of interest to notice that body cells are not possessed of any complex means of propelling themselves, such as the walking of man, the ciliary movements of many bacteria and the paramecia. They move by liberating a surface-tension-lowering substance. This substance is peculiar in that it fails to decrease the surface tension of water, but is specifically absorbed only by the primary food constituents of the cell, proteins and fats and substances having like affinities (5). A cell liberating this substance into a medium draws the mobile proteins and fats to it. It is drawn into fixed masses of proteins and toward larger masses of fat, which have a greater inertia than the cell. It became evident, therefore, that coal tar may act to build a dense mass of cells capable of cancerous growth through its ability to draw cells to it away from their blood vessels and intercellular substances. Such a dense mass of cells must sooner or later acquire the property of growth and increase in mass.

Jorstad (10), working in the laboratory, has studied the effect of coal tar in the tissue. He finds it acts alone in this capacity. Drops of coal tar collect the cells from the surrounding tissue. They absorb and become saturated with the surface-tension-lowering substance of these cells. They do not stimulate the cells to grow. Growth intervenes when the mass of cells about the drop of tar reaches a certain density. Such a mass of cells can be accumulated by the tar only when it is placed in a cellular tissue. In a less cellular tissue, as the normal subcutaneous connective tissue of adult rats, too few cells are collected often for growth to intervene. These few cells not only fail to show growth, but shrivel as their lipoid soluble substances are lost to the tar.

THE ACTION OF BACTERIA AND THE NORMAL STIMULUS FOR LIFE IN THE BODY

According to the formulation presented by the above observations, growth is dependent on the presence of a certain concentration of a growth stimulus about the cells. This growth stimulus is formed by the cells normally when they are crowded together in a stagnant environment and supplied with oxygen. I have shown that this is true for the cells in the body as well as in the cultures. In the cultures it has been noticed that this stagnation must be excessive for growth to intervene. It must be more excessive than the stagnation which exists about the cells in most parts of the young child. This suggests that special means are necessary for supplying this substance to the cells of the child in order that it may grow. From the study of the nutrition of children, it has been shown that certain substances, aside from proteins, fats and carbohydrates, are essential for growth. These other essential substances are the vitamines. Two important vitamines are designated as A and B. A is fat-soluble, B is water-soluble. It has been shown above that the cells migrate by liberating a fat-soluble substance. Coal tar absorbs this substance in drawing the cells to it. The cells suffer by the loss of this substance to any more than very small quantities of tar injected subcutaneously. Larger quantities of tar lead always to the emaciation and death of the animals. Noting the relation between the growth stimulus, the *archusia* and vitamine B and this fat-soluble substance of the cell and vitamine A, it became

of interest to see what relation they may have to each other.

Jorstad investigated then the toxicity of coal tar and the relation of vitamine A in counteracting it. He finds the toxicity of the tar is readily counteracted by feeding butter-fat and cod liver oil. These substances contain vitamine A.

Bacteria, as it is known from the work of Edwin Smith (11), must induce the cancerous formation in plants not through drawing the cell to them, but through inducing an active local growth of a few cells. They produce thus the same cancerous organization, not by collecting cells as the drops of coal tar produce it, but by stimulating the growth of a few cells. We have investigated these organisms in relation to vitamine B and find them to be readily substituted in the diet of animals for vitamine B, but not for vitamine A. When fed they allow and stimulate the growth of the animal. When injected locally they stimulate the growth of the cells about them and produce a cancerous organization.

Function, we have found in the study of heart muscle cells, is the result of polarizing the cells. This polarization is brought about by the growth of blood vessels between them. This growth of vessels hinders and prevents growth. Function is something which has developed at the expense of the growth mechanism in the cell. The body is able to grow only as it is able to acquire a part, at least, of its growth-stimulating substances from other growing plants and animals. Our high functional state, as is evident, therefore, has been made possible only through an evolutionary development. Higher forms of animal life exist as such only as they can acquire, in part, at least, the life energy from lower growing and non-functioning forms.

We have also sought a source for this stimulus in other parts of the body. It is well known that the glands of internal secretion play a definite rôle in promoting growth. The hypophysis acts in this capacity on the skeleton. The sex glands liberate substances which act on the general metabolism of the organism. It thus became of interest to study directly the effect of the secretion of these organs on the growth of cells in general. Recently, Allen and Doisy (12) isolated the active hormone of the ovaries. Johnston and the writer (13) have been studying the action of this hormone by a newly devised method. Inert fats, such as mazola or corn oil, have been injected into the subcutaneous tissue of rats. Droplets of these fats remain in the tissues for a long time, becoming encapsulated by a collar of fibroblasts, much as coal tar becomes encapsulated. In other experiments, ovarian hormone was added to the fat. The connective tissue cells invaded the oil, which contained the hormone, grew most actively and consumed it. These cells digested this fat, as the cells of the culture digest the fat, either after they have accumulated the *archusia* about them, or it has been added to the medium. These latter facts have a significance not only in showing directly why the woman accumulates fat at her menopause, but for explaining the development of cancer at this period. It is a well known fact that if one bud of a limb be stimulated, the others atrophy. Cancer, as I have described it, is a normal growth of cells, which becomes abnormal only because of its location, its eventual destruction of its own circulation and the associated necrosis and its abnormal fermentative processes. In an actively stimulated organism such growth must proceed with difficulty. For the cancer to grow it must produce a great amount of energy in order to overcome the normal resistance of the tissues about it. It is therefore apparently not unusual that cancer should be a disease of the aged. It must be much more easily produced at the period of decline or when growth energy is much lessened in the body as a whole.

CONCLUSION

No attempt has been made as yet to investigate the action of X-ray and radium. It has long been known that these rays may not only cure cancer, but induce its forma-

tion. It has been shown above that the same stagnation and cell-crowding which leads to growth will in a greater degree lead to the destruction of the cells. Wolbach (14), several years ago, showed that the rays of X-ray act on the blood vessels to destroy them as such. Recently Steenbock and Black (15) have shown that the rays from a quartz mercury lamp will liberate growth-promoting substances in liver and muscle of rats.

As I have just stated, we have not studied as yet the action of the rays of radium and X-ray. The object of this report has been to bring facts before you which may add materially in making such analysis and which I feel show quite definitely that cancer is not a disease in the sense of the infectious diseases, but that it is the normal reaction of body cells in a particular environment. It is to be classed, therefore, with gangrene and atrophy. Gangrene became understood when the necessity of oxygen became understood. Gangrene results from any condition which stops the flow of blood to a part. Cancer comes into existence when the normal arrangement of cells and blood vessels is disturbed, so that the cells become sufficiently crowded in an area of reduced circulation. These conditions lead, in the presence of oxygen, to the formation or the accumulation of an active growth-stimulating substance. The eventual cure and prevention of this condition will be made, therefore, through the use of quantitative changes in the whole—and not qualitative ones. These necessary changes will become known through the more exact knowledge of those substances and conditions, known to be able to produce and also destroy cancer, such as X-ray, radium, etc.

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DISCUSSION

Dr. Edwin C. Ernst (St. Louis): This interesting newer phase and line of attack upon the cancer problem certainly merits a most critical and careful consideration. I wish to state at this time that I am grateful to Dr. Burrows for having had the opportunity of working with him at the Barnard Free Skin and Cancer Hospital, and being thus, by co-operation, likewise allowed the privilege of helping to analyze or establish facts as to the effects of X-ray and radium radiations upon body structures, and their relation to cell reactions, beginning with the very first normal tissue changes and extending to the period when definite cancer has been established.

The problems confronting us are many, although some of these investigations are comparatively simple because the bio-chemical, normal and pathological foundations or groundwork have been so carefully and interestingly developed and described here by Dr. Burrows. Nevertheless, we realize the difficulties confronting us in attempting to further develop and more thoroughly understand the theoretical considerations as

they have been presented, especially the possible relationship of the various types of radiant energy to normal and pathological cell changes, both from the scientific laboratory and the clinical standpoints. The probable actual close relationship of the human and animal cell phenomena is the second great problem which confronts us and has been the most difficult stumbling block and barrier between animal experimentation in cancer research and the human clinical phenomena. This wall or barrier is frequently longer and higher than the old Chinese wall, in fact so much so that successful animal experimentations are at times completely isolated and thus are found to be of no practical value. Human and animal tissue actions or reactions to cell stimulation are frequently at variance and do not check. The definite actions or reactions of the cells in the one cannot be duplicated in the other.

The experimental phenomena described and discussed here certainly appear to be fundamental. Cell growth actions and reactions follow normal and pathological irritations and stimulations with a uniformity that certainly suggests the probability of an unusually close relationship between the two, although there will always be certain fundamental differences.

Normal phenomena of human and animal experimentations throughout the early stages are merely a matter of normal and abnormal cell arrangements or disarrangement.

The findings certainly warrant the assumption that there is a direct and indirect effect upon the intra- and extracellular fluid constituents of the tissues under the influence of specific quantities of absorbed X-ray or radium energy. Our great problem, therefore, may prove to be simply one of applying a quantity of radiant energy sufficient to control these complex substances. Such changes apparently did bear a direct relationship to the cell and its surrounding structures whenever the fluid concentrations were either increased or decreased, and thus were found to be either beneficial or harmful to either the normal or abnormal pathological cell groups.

Therefore, we made every effort to standardize the introduction of radiant energy of X-ray into the deeper tissues of the body and adopted technical methods applicable to the higher, medium or the lower voltages of our machines for both the human and the animal experimentation, which methods of measurement are, we believe, and in fact have found them to be, a practical means of duplication of the respective higher or lower voltages of X-ray energy within minimum limits of error in dosage, thus controlling the radiant energy absorption.

There is nothing new or original about the method which I hope I may find sufficient time to describe before this body. The mechanical and physical features have been simplified rather than complicated, nevertheless they are similar to the usual methods of measurement adopted by the trained physicists. I present them to you as installed at the Barnard Free Skin and Cancer Hospital, and thus far they have proven to be very practical—almost foolproof—yet admirably efficient in every way.

As stated above, the energy can be accurately duplicated from day to day and from month to month.

We all realize after what has been stated at this meeting that in order to intelligently report our results, the amount and quality of X-ray administered must in every case be capable of duplication, and they must be intelligently understood by each and every radiologist. Most important of all is it that the dosage should be both accurate and possible to duplicate. We all realize that variations in doses must of necessity produce different physiological and biological effects.

Conditions beyond our control do change the radiation output of our transformers, perhaps without our knowledge or that of our assistants. Any method of operation is valueless unless repeated checks or controls can be employed, so that the X-ray energy output will not only be uniform from hour to hour, but also from year to year.

Personally, I have found and continue to find it necessary to repeatedly eliminate mechanical and physical sources of error in our installation, since the ordinary sphere gap measurements, milliampere readings, etc., are indeed very limited by themselves in their respective valuations in relation to the changes in the output of our machine, in the hands of the ordinary radiologist or assistants employed. However, we should always take into consideration the fact that the sphere gap voltage factors, milliampere readings, etc., are not sufficiently accurate by themselves, but that, in addition, the direct output of radiant energy should also be checked. Ionization methods, controlled by known and definite amounts of radium or other supplementary methods or system of measurements, should be employed as a check so that the quantitative tissue absorption can at least be approximately measured.

The water-cooled tube demands still greater accuracy as to dosages, and added precautionary measures must necessarily be instituted because of the greater volume of X-ray energy employed within a shorter period of time.

In conclusion, therefore, I wish to emphasize the very important essential requisite of correct dosage readings of quantity and quality in both our animal and human experimentations and practical X-ray and radium treatments, and the probable influence of even slight variations in dosage upon the phenomena of normal or abnormal tissue growths.

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Die Bewegung des Epithels der Haut.

Von

Montrose T. Burrows.

Mit 5 Abbildungen im Text und Tafel 6—7.

Die Amöbe bewegt sich mit einer unregelmäßigen Schwimmbewegung ihres Zytoplasmas. Sie streckt Pseudopodien aus, mit deren Hilfe sie sich an feste Strukturen im Substrat hängt. Der übrige Teil des Zytoplasmas fließt dann in diese Pseudopodien hinein. Diese Bewegungen scheinen nach der übereinstimmenden Meinung der meisten Autoren aus dem Innern der Zelle ihren Ausgang zu nehmen. Die Amöbe kann ihre Pseudopodien ebenso in einer Flüssigkeit ausstrecken, wie wenn sie an ein Substrat gesetzt ist. Diese Bewegungen erfolgen in keiner geraden Linie, sondern nehmen einen unregelmäßigen Kurs. Es ist auch möglich, daß die Amöbe unter gewissen Umständen sich durch Krümmung des Körpers gegen ein starres Substrat fortbewegen kann.

Es ist zwar richtig, daß die meisten Beobachter schon seit langer Zeit angenommen haben, daß Körperzellen eine ähnliche Art Fortbewegung haben wie die Amöbe und darum auch vermutet haben, daß die Zellen ein Entoplasma, ein Ektoplasma und auch eine äußere Membran besitzen, es wurde aber dennoch kein direkter Beweis zur Unterstützung dieser Anschauung erbracht. Wenn wir viele verschiedene Körperzellen in der Gewebekultur beobachten, so finden wir, daß diese aus nicht viel mehr bestehen als aus einem einfachen flüssigen Protoplasma, in welchem ein Nucleus schwimmt,

aus Centrosomata, aus Mitochondrien, aus Fett und vielleicht aus anderen geformten Proteinen und Fettkörperchen. Wie ich schon im Jahre 1913 (1, 2) gezeigt habe, sind auch ihre Bewegungen kaum verschieden von denen der Amöbe und wahrscheinlich die Folge eines viel einfacheren Mechanismus. Gleich im Beginn meiner Arbeiten mit Gewebekulturen habe ich schon bemerkt, daß die Körperzellen sich direkt herausbewegen und sich von dem Fragment des Gewebes entfernen, das in einem Tropfen Kulturmedium sich befindet. Diese Bewegung vollzieht sich entlang den Diffusionslinien, die bei allen anderen Substanzen beobachtet werden, nämlich vom Fragment in das Medium oder von dem Medium in das Fragment. Die Fortbewegung der Zellen findet meistens ohne irgendeine amöboide Bewegung statt, im Gegensatz zu der öfters ausgesprochenen Behauptung, daß Zellbewegung immer mit amöboider Bewegung verbunden ist. Sehr oft bewegen sich aktive, normal wachsende Zellen lange Strecken entlang, ohne die kleinste Veränderung in ihrer Kontur zu zeigen. Diese Zellen bewegen sich ebenso wie andere Körper, die Oberflächenspannungs-erniedrigende Substanzen frei werden lassen. Wenn Pfefferpulver an die Oberfläche von Wasser gestreut wird, so schnellen die einzelnen Granula voneinander, um gleichsam an der Wasseroberfläche verteilt zu werden. Diese Bewegung der Pfeffergranula ist die Folge des Freiwerdens einer Oberflächenspannungs-erniedrigenden Substanz. Diese Substanz hat einen bestimmten Sättigungsgrad entsprechend ihrer Löslichkeit in Wasser. Diese Granula bewegen sich viel schneller an der Wasseroberfläche des reinen Wassers, als an der Oberfläche eines solchen Wassers, das schon zum Teil mit dieser Oberflächenspannungs-erniedrigenden Substanz gesättigt war, und sie bewegen sich überhaupt nicht an der Oberfläche des Wassers, wenn dieses vorher gänzlich gesättigt war.

Wenn ein Stückchen Milz in eine dünne Schicht von Blutplasma gelegt wird, welches von einem Deckgläschen in die feuchte Kammer eines ausgehöhlten Objektträgers hängt, so wandern die Lymphozyten, Leukozyten und roten Blutkörperchen und die fixen Bindegewebezellen aus in das Medium. Die Bewegung der Zellen fängt nicht auf einmal an, sondern immer nach einer gewissen Latenzperiode. Diese Latenzperiode variiert mit der Wachstumsaktivität und mit dem Alter des Gewebes und ist in den einzelnen Zellarten verschieden. Aus einem Milzfragment eines eben ausgebrüteten Hühnchens beginnt die Auswanderung der Lymphozyten und Leukozyten schon nach 2 Stunden, dagegen

erscheinen die Bindegewebszellen erst nach 24 Stunden oder 5—6 Tagen.

Ohne die verschiedenen Typen der Wanderzellen zu besprechen, die aus diesem Fragment sichtbar werden, ist es von Interesse zu bemerken, daß die ersten Zellen, die herauswandern, sehr klein sind, in Vergleich mit denen, die später kommen. Diese kleinen Zellen kommen früh heraus und bilden einen dichten Hof um das Fragment, bald erscheinen große ovale Zellen und dann spindelförmige Bindegewebezellen. Der dichte und schmale Ring um das Fragment, bestehend aus kleinen Wanderzellen, wird breiter, wenn die andern großen Zellen erscheinen, und verliert naturgemäß an Dichtigkeit der Zellmasse. Die einzelnen Zellen in diesem Ring fahren fort, sich aus dem Fragment herauszubewegen, bis sie entweder gleich verteilt oder in die fernsten Ecken des kleinen Plasmatropfens zerstreut werden. Die großen ovalen Zellen und Fibroblasten bewegen sich in einer geraden Linie. Diese Bewegung bleibt nur für eine kurze Zeit bestehen und hört dann auf. Um die Fragmente von Bindegewebe und embryonalem Mesenchym herum bewegen sich die Zellen in jeder Richtung, als lange Reihen von Zellen; diese Bewegung beginnt nach einer Latenzperiode. Sie ist sehr aktiv im Anfang, später aber wird sie verlangsamt, nachdem eine gewisse Menge Zellen ausgewandert sind.

Ich studierte die Wanderung der Leukozyten, Fibroblasten, des Mesenchyms und des embryonalen Herzmuskels. Während jede der Zellen eine Formveränderung aufweisen kann, welche einer amöboiden Bewegung ähnlich ist, bewegen sich die meisten lange Strecken ohne Änderung. Kein Beweis besteht, daß die Zellen kriechen können in irgendeinem Sinne des Wortes, sondern sie scheinen von einer Kraft angezogen zu werden, die verschieden ist von der sichtbaren mechanischen Struktur. In dem Falle der Mesenchym- und Herzmuskelzellen waren die Unregelmäßigkeiten der Form von Unregelmäßigkeiten des Mediums begleitet.

Während die Wanderung der Zellen augenscheinlich eine gewisse Ähnlichkeit hatte mit der Bewegung der Pfefferkörnchen, so waren doch gewisse Eigentümlichkeiten vorhanden, die den Gedanken nahe brachten, ob nicht ein anderer Mechanismus übersehen wurde. Eine der Haupteigentümlichkeiten besteht darin, daß die Zellen sich nicht in eine einfache Salzlösung hineinbewegen oder auf der Oberfläche einer solchen Lösung bewegen können. Sie können nur in ein festes Medium hineinwandern, welches Eiweiß, Fett oder andere Substanzen enthält, die eine gleiche Affinität zu Lipoiden haben.

Diese Tatsache schließt nicht die Möglichkeit aus, daß ihre Bewegung durch Oberflächenspannungsveränderung verursacht wird. In wässerigen Lösungen können Körperzellen sich deswegen nicht fortbewegen, weil sie keine Substanz hervorbringen, welche die Oberflächenspannung von Wasser erniedrigt. Sie bewegen sich dagegen in die Proteine und Fette, weil sie solche Substanzen abgeben, die sehr leicht von diesen absorbiert werden.

Zum Beweis dieser letzteren Möglichkeit habe ich durch direkte Analyse von embryonalem Mesenchym, Muskel und von erwachsenen Tieren stammenden Bindegewebekulturen gefunden, daß sie gleichzeitig eine Substanz freisetzen, die sehr leicht durch das Fibrinogen das Plasmas und durch die Fette absorbiert wird. Diese Substanz kann sehr gut erkannt werden dadurch, daß sie das Fibrinogen zu Fibrin koaguliert und die Mesenchym- und Bindegewebezellen an dieses heftet. Fett in Kontakt mit den Zellen, oder in die Zellen aufgenommen, wird mit dieser Substanz gesättigt. Diese Zellen wandern nur dann in die Plasmaschicht, wenn die letztere dem Deckglas fes ansitzt. Falls aber das Plasmagerinnsel nur lose angeheftet is so wandern die Zellen nicht aus, sondern kleinste Teilchen des Plasmas, bestehend entweder aus Protein oder Fett, werden in das Gewebsstück hineingezogen. Das gesamte Gerinnsel wird wie von einem Magneten an das Gewebsstück herangebracht.

Die Form dieser Zellen ist nicht durch ihre innere Organisation bestimmt, sondern durch diese Substanz, welche die Gerinnung des Fibrinogens verursacht und Fibrillen erzeugt, an welchen die Zellen haften. Wenn das geronnene Plasma gespannt ist, so wird das Fibrin in lange Fibrillen gezogen. Die an die Fibrillen angehefteten Zellen nehmen eine Spindelform an. Wenn das Plasma wie eine Schicht gerinnt, so werden sie platte polygonale oder spindelförmige Zellen an dieser Oberfläche. Wenn ein Netz sich formt, so werden die Zellen sternförmig. Suspendiert in eine Flüssigkeit, nehmen sie eine runde Form an, wie irgendein anderer Tropfen einer nicht mischbaren Flüssigkeit sich unter denselben Verhältnissen abrundet.

Diese Substanz, die durch die Bindegewebszellen freigesetzt wird, habe ich Ergusia oder die arbeitende Substanz der Zellen benannt. Andere Zellarten können eine ähnliche Ergusia freisetzen, oder eine, die etwas andere Eigenschaften hat (3, 4). Die durch die Leukozyten und Lymphozyten freigesetzte Substanz koaguliert das Fibrinogen nicht zu echtem Fibrin, sondern mehr zu einem Gel. Diese Zellen wandern in das Gel durch schmale Kanäl-

chen, welche durch die Lösungseigenschaft der Zellen entstehen. Sie haften nicht fest an dem Gerinnsel, sie nehmen in diesem eine sphärische Form an. Ihre Pseudopodien sind meistens stumpf und abgerundet. Die Epithelzellen der Haut bewegen sich als Membran aus dem Fragment heraus. Sie unterscheiden sich von dem Bindegewebe dadurch, daß die Zellen nur selten auseinanderweichen. Es ist von Interesse zu bemerken, daß sie im Tierkörper keine intercellulären Fibrillen bilden, was bei Bindegewebszellen sehr gewöhnlich ist. Bei dem Studium der Bewegung und der Bildung von Epithelialmembranen aus der Haut von Embryonen, aus Drüsen und aus der Haut von erwachsenen Fröschen habe ich bemerkt, daß diese Zellen das Plasma koagulieren und sehr ähnlich, wie die Bindegewebszellen in dem Gerinnsel haften. Der Unterschied besteht darin, daß diese Koagulation von einer Auflösung gefolgt wird, ebenso wie das Kasein zuerst im Magen ausgeflockt und dann verdaut wird. Die äußeren Zellen der Membran haften an dem geronnenen Plasma und bewegen sich vorwärts. Hinter diesen Zellen wird das Gerinnsel sofort aufgelöst. Die Membranen werden durch diese Vorwärtsbewegung der äußeren Zellen gebildet. Es ist dies die ausgespannte Membran des Fragments. Diese Bewegung wird nur bis zur Grenze der Elastizität der ursprünglichen epithelialen Membran fortgesetzt, dann löst sie sich von dem Gerinnsel, reißt in ihrer Mitte, wenn das Fragment vorwärts gezogen wird (5). (Siehe Burrows 6, Fig. 1 und 6.)

Ein anderer Unterschied zwischen der Bewegung der Zellen und derjenigen der Pfefferkörnchen ist die Latenzperiode und das Ausbleiben der völligen Dispersion aller Zellen des Fragments. Während Pfefferkörnchen die Oberflächenspannung erniedrigende Substanz sofort freisetzen, wird die Ergusia von Gewebszellen erst dann abgegeben, wenn ein gewisses primäres Produkt der normalen Oxydation dieser Zellen sich zu genügender Konzentration angehäuft hat (7). Diese Substanz ist sehr leicht von den wachsenden Geweben mittels Salzlösung zu extrahieren. Es kann gezeigt werden, daß sie die Zellen entsprechend ihrer Konzentration verschieden beeinflußt. Ich habe diese Substanz Archusia (*S*) oder die treibende Substanz der Zelle genannt. In schwacher Verdünnung (S_1) hat sie keinen Effekt, in größerer Konzentration (S_2) stimuliert sie die Zelle zum Wandern und zur Aufnahme von Proteinen, Fetten und anderen Substanzen. In noch stärkerer Konzentration (S_3) veranlaßt sie die Zelle zu wachsen und sich zu teilen. Noch stärker konzentriert (S_4) führt sie zur Selbstverdauung der Zelle (8).

Um eine für die Wanderung genügende Konzentration der Archusia zu bilden, muß die Menge des Mediums klein sein, die der Zellen verhältnismäßig groß. Wenn eine noch größere Anzahl von Zellen in einer kleinen und angestauten Menge eines Mediums supendiert wird, dann tritt Wachstum ein. Ist die Anzahl der vorhandenen Zellen nicht groß genug, dann muß Archusia von anderen Quellen extrahiert und hinzugefügt werden, um Wachstum zu ermöglichen. Diese Wanderung beginnt also nach einer Latenzperiode, die nötige Periode für die Anhäufung der Archusia. Sie muß aufhören nach einer gewissen Ausbreitung des Fragmentes und Zerstreuung der Zellen, oder Verdünnung der Archusia, die durch die begrenzte Menge der anwesenden Nahrung gebildet wird.

Wenn wir diese Tatsachen auf den Körper übertragen wollen, ist es von Interesse, daß man eine Stauung des Kreislaufes findet in dem Bereiche von aktivem Wachstum, so wie im Knochenmarke und in den Fingernägeln. Die Wucherung in den Wunden folgt genau der Verteilung der Entzündung, die den Kreislauf verlangsamt und der vorherigen Zerstörung der Blutgefäße. Der Krebs ist eine dichte stagnierende Zellmasse. Wir haben gefunden, daß die Substanzen oder die Verhältnisse, die zu Krebs führen, z. B. Teer, tierische Parasiten, Bakterien usw., nur dadurch wirken, daß sie eine Zellmasse bilden. Einmal ausgebildet, wächst sie weiter und bildet vermittels der Zerstörung von Blutgefäßen immer von neuem eine stagnierende Zellmasse (9, 10, 11).

Diese Beobachtungen führten mich daher zu dem Schlusse, daß die Zellen einfach flüssige Systeme sind, dazu befähigt, in sich selbst gewisse Reaktionen mit Sauerstoff hervorzurufen. Diese Reaktionen setzen außer Wärme gewisse Substanzen frei, die Archusia. Die Archusia verursacht die Entstehung der Ergusia. Die letztere führt zur Auswanderung der Zellen in das Medium, das fixiertes Protein und Fett enthält oder aber führt zur Anziehung der mehr beweglichen Proteine und Fette in die Zelle hinein. In höherer Konzentration veranlaßt dieselbe Substanz Verdauung des Eiweißes und Fettes und zum Wachstum. Wasser und Salze gelangen in die Zelle als Folge dieser sekundären Reaktionen. Die Wanderung und die Zellformen sind nichts anderes als eine einfache Oberflächenspannungserscheinung, wie oben erwähnt (3). In der Literatur finden sich widersprechende Meinungen. Oppel (12) und Osowsky (13) studierten die Bewegungen epithelialer Membranen von Fragmenten der Cornea und von Embryonen. Sie

bemerkten, daß diese Zellen sehr oft ohne jede amöboide Aktivität sich bewegten. Sie nahmen an, daß diese Bewegung durch die Zellen bewirkt wird, aber sie nannten dies „Massenbewegung", nicht amöboide Bewegung. Holmes (14) vertritt eine andere Ansicht. Er demonstrierte zarte amöboide protoplasmatische Fortsätze entlang der Grenze einer epithelialen Membran, die sich in einem gelatinösen Medium in Bewegung befand. Seiner Ansicht nach senden die Zellen diese Fortsätze aus, etwa wie ein Mensch seine Beine bewegt.

Ein Studium der Abbildungen von Holmes legt die Vermutung nahe, daß diese Unregelmäßigkeiten der Oberfläche auf das im Medium befindliche Gelatin zurückzuführen sind, weil dieses von den aus den Zellen freigesetzten koagulierenden Substanzen verändert wird. Demnach bedeuten die Zellfortsätze entweder eine Trennung des Gerinnsels in Fibrillen oder eine Unregelmäßigkeit der Berührungsfläche, aber nicht eine durch die Zellen bedingte amöboide Bewegung.

Um auch diese Frage zu beleuchten, habe ich Hautkulturen von erwachsenen Fröschen angesetzt. Als Medium benutzte ich aus dem Blute des erwachsenen Frosches hergestelltes Plasma. Das Hautfragment war von dem Beine desselben Tieres genommen. Vor der Entfernung wurde die Haut sorgfältig mit Wasser und Seife und dann mit sterilem, 0,4% Toluol enthaltendem Wasser gereinigt. Die Fragmente enthielten die epitheliale Schicht und einen Teil der oberflächlichen Fascie. Jedes Fragment hatte einen längsten Durchmesser von einem Millimeter oder weniger und wurde in eine Schicht von Plasma von 0,5 mm Dicke oder weniger gelegt. Diese Dimensionen sicherten genügende Sauerstoffzufuhr.

Das Plasma wurde auf ein Deckgläschen gelegt, darüber wurde ein ausgehöhlter Objektträger gelegt, welcher vorher mit Vaselin umrandet wurde. Die Kulturen wurden in einem Zimmer gehalten, dessen Temperatur von 65–70° F variierte. Die Epithelzellen beginnen nach 2 Stunden ihre Auswanderung bei 70° F. Bei einer niedrigeren Temperatur sind die Bewegungen langsamer. In einem Teile dieser Kulturen bewegten sich die Epithelzellen an der Berührungsfläche des Plasmas und des Deckglases. In anderen Kulturen dagegen war diese Schicht in den unteren Teil des Gerinnsels gelegt worden, so daß sie sich auf der freien und wenig unterstützten Plasmaluftoberfläche bewegten.

Kurz vor und während der Zellbewegung zieht sich die Plasma-Schicht zusammen, indem das Fibrinogen sich zu Fibrin umwandelt.

Fig. 1.

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M T. Burrows.

Fig.

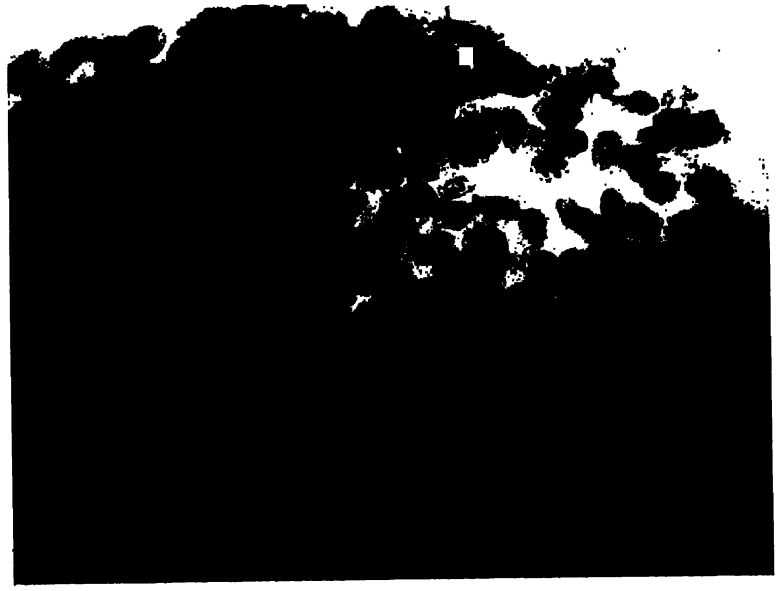

Fig. 8

Verlag von Gustav Fischer in Jena.

M. T. Burrows.

Diese Umwandlung beginnt an der Grenze der Epithelmembran. Gleichzeitig flachen sich die Epithelzellen ab und heften sich an das Fibrin (Fig. 1, siehe Tafel Nr. 6). Während nun der Gerinnungsprozeß zentrifugal weiterschreitet, wandern die peripheren Epithelzellen aus und ziehen die anderen Zellen der Membran nach sich. Wenn die Epithelzellen dem Deckgläschen nahe liegen, kontrahiert sich das Plasmagerinnsel zu einer flachen Schicht. Die Zellen gleiten über diese glatte Fibrinschicht ohne irgendwelche Konturveränderung (Fig. 2). Wenn dagegen die Zellen über die

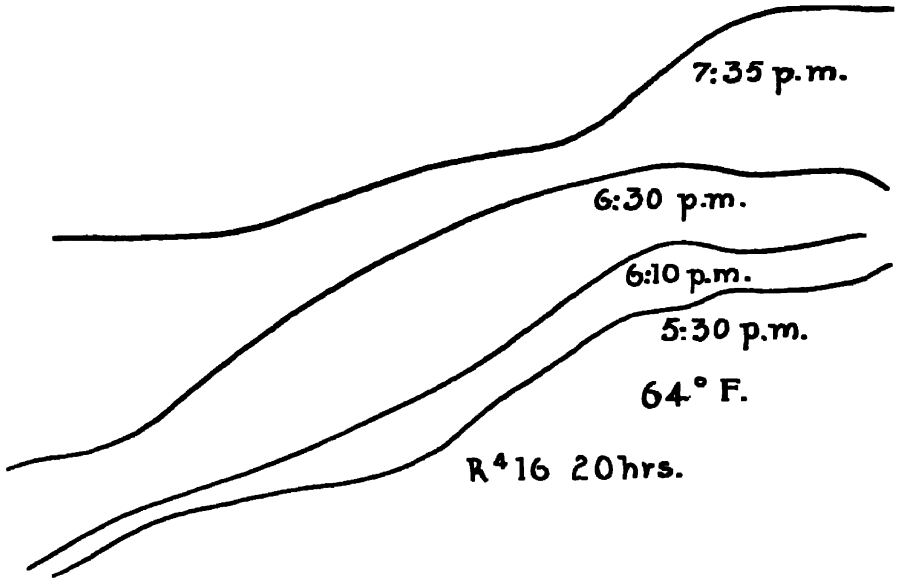

Fig. 2. Eine Camera lucida-Zeichnung vom Rande einer epithelialen Membran bei starker Vergrößerung. Die Zeichnung wurde eine halbe Stunde später angefertigt, nachdem die Membran sich von dem Fragment getrennt hat. Die epitheliale Membran stammt von einem Fragment der Haut eines erwachsenen Frosches. Sie hat sich in die Nähe des Deckgläschens bewegt.

freie Oberfläche des Gerinnsels wandern, ändert sich das Bild. Die Spannung der sich ausbreitenden Epithelmembran zieht die Oberfläche in feine Fibrillen aus. Die Zellränder werden dann gezackt. Diese Unregelmäßigkeiten der Zellränder werden also durch Unregelmäßigkeiten der Oberfläche des Mediums hervorgerufen.

Je mehr sich die Epithelmembran ausdehnt, um so größer ist der Zug an der Plasmaoberfläche und um so stärker die Bildung von Fibrillen. Dadurch werden wieder die Konturunregelmäßigkeiten der Zellen größer und ausgeprägter (Fig. 3).

Wenn der Spannungsgrad noch mehr zunimmt, dann löst sich die Membran teilweise von dem Medium ab; die Zellausläufer sind dann sehr lang (Fig. 4) und bestehen oft aus zusammenfließenden Zytoplasmen mehrerer Zellen.

In anderen Kulturen sieht man große Unregelmäßigkeiten an diesen sich bewegenden Membranen. Wie ich schon in meiner

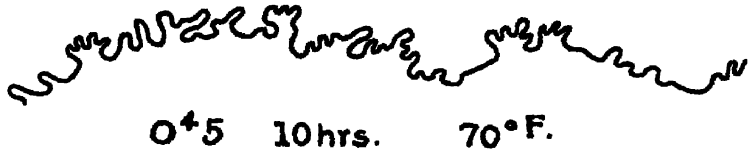

Fig. 3. Eine Camera lucida-Zeichnung von schwacher Vergrößerung einer ähnlichen Membran, welche aus dem Fragment sich ausstreckt. Der Rand dieser Membran haftet an der unteren Fläche des hängenden Tropfens. Sie ist schon breit und dicht und übt starke Spannung auf das Gerinnsel aus. Das Gerinnsel ist in gröbere Fibrinfibrillen verwandelt. Entsprechend dieser Veränderung in dem Gerinnsel sieht man breite Ausläufer am Rande der Membran.

anderen Arbeit nachgewiesen habe, können sich diese dünnen Membranen nur bilden, wenn das Fragment und das Gerinnsel fest geheftet sind. Wenn das Gerinnsel nicht haftet, so wird es „en masse" zu dem Fragment gezogen und aufgelöst (Burrows 6, Fig. 1). Wenn das Fragment sich bis auf einen Rand ablöst, so

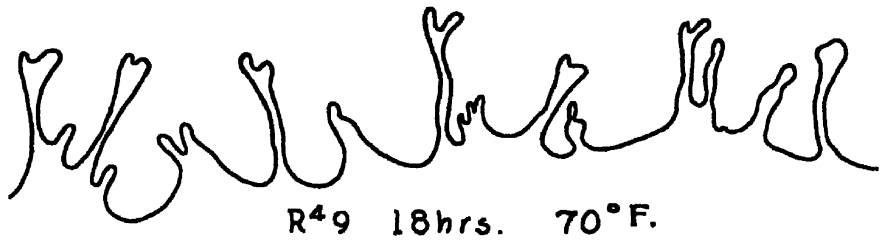

Fig. 4. Eine Camera lucida-Zeichnung von einer schwachen Vergrößerung des Randes einer epithelialen Membran, die bis zur Grenze ihrer Elastizität gespannt ist. Siehe die langen Ausläufer der Zellen oben bevor sie sich von dem Gerinnsel abtrennen sollten.

wird es in das Gerinnsel wandern und sich einen Weg in dieses brechen, ohne eine Membran zu bilden. Es ist interessant zu bemerken, daß da, wo das Gerinnsel unregelmäßig an dem Deckgläschen haftet, sich die Membranen viel aktiver gegen den haftenden Teil bewegen als gegen den losen Teil. Es erscheinen an dem Rande gröbere und feinere Unregelmäßigkeiten (Fig. 5 und 6).

Fig. 7 und 8 sind Photographien zweier anderer Membranränder, welche die feinen Zähnelungen der Zellen zeigen und die entsprechenden Unregelmäßigkeiten in dem Medium. Die Zähnelungen sind identisch mit den von Holmes abgebildeten, die dieser mit amöboiden Ausläufern verglichen hat. Wenn man den Rand der in

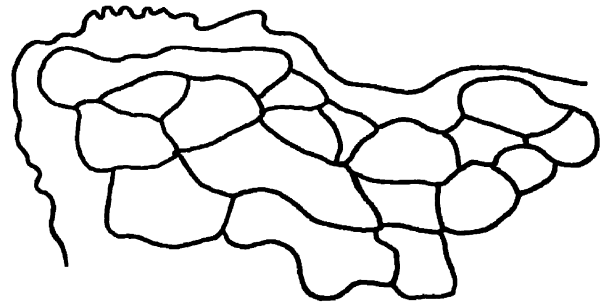

Fig. 5. Eine Camera lucida-Zeichnung des Randes einer epithelialen Membran, wie sie sich in die untere Oberfläche des Gerinnsels bewegt, welches unregelmäßig an dem Deckgläschen haftet. Wo das Gerinnsel fest anhaftet, hat sich diese Membran am weitesten fortbewegt.

Fig. 7 (siehe Tafel Nr. 7) dargestellten Membran betrachtet, so sieht man um den Rand der epithelialen Membran herum und etwas entfernt darüber hinaus einen umgrenzenden schleierartigen Kranz. Dieser bezeichnet den Bezirk des kontrahierten Fibrins. Unter diesem Bezirk hat sich Serum angesammelt, auf dem Grund

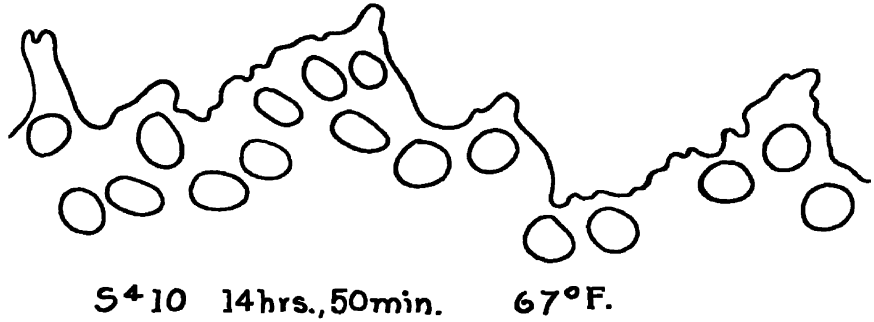

Fig. 6. Camera lucida-Zeichnung des Randes einer epithelialen Membran, kleinere und größere Unregelmäßigkeiten zeigend, als Folge unregelmäßigen Anhaftens des Gerinnsels an dem Deckgläschen.

des Gerinnsels. An der Peripherie der Membran sieht man, daß das Gerinnsel mit kleinen Serumtröpfchen gefüllt ist. Diese haben verschiedene Gestalt. Einige sind verlängert, andere klumpenförmig, oval oder rundlich. Jedes hat eine Fibrinwand; ein Zellausläufer streckt sich in diese Wand hinein (siehe Fig. 8, Tafel Nr. 7).-

Fig. 8 ist eine Photographie von dem Rande einer anderen Membran derselben Kultur, aus welcher Fig. 7 angefertigt wurde. Eine sorgfältige Beobachtung mehrerer solcher Membranen zeigt, daß diese Ausläufer immer den primären Veränderungen des Gerinnsels entsprechen. In diesem Falle sind diese offenen Räume sehr breit. In solchen Fällen werden die Unregelmäßigkeiten in dem Membranrande von einer oder von mehreren Zellen gebildet. Diese Unregelmäßigkeiten sind nur Verzerrungen, welche auch andere Flüssigkeitstropfen zeigen, wenn sie eine Oberflächenspannung erniedrigende Substanz in eine ungleichmäßige Absorptionsoberfläche abgeben.

In einer Kultur von der Haut eines Hühnerembryos spielt noch eine andere Erscheinung eine Rolle. Die zentralen Teile dieser mehr zellulären Fragmente werden zu einer flüssigen Substanz abgebaut, welche die Oberflächenspannung des Wassers erniedrigt. Diese Substanz bildet eine Schicht über der Wasseroberfläche. Die Zellen des Fragmentrandes wuchern in diese Schicht hinein. Die Fähigkeit embryonaler Gewebe, diese Schicht zu bilden, macht es möglich, diese im flüssigen Medium zu kultivieren und deren Wanderung in des Medium zu beobachten. Diese Zellen bewegen sich nicht entlang dem Deckgläschen oder der freien Oberfläche der Salzlösung, wie es sich Harrison vorgestellt hat, sondern in diese dünne Schicht hinein, welche den wachsenden Zellen vorauseilend sich über das Medium ausbreitet. In einigen Kulturen der Froschhaut habe ich ähnliche Schleier gesehen; in den Schichten von gleichmäßiger Konsistenz ist der Rand der wachsenden epithelialen Membran oft viel gleichförmiger und ohne amoboide Ausläufer.

Holmes schildert in großen Zügen diese Vorgänge, die ich in Fig. 4 gezeigt habe. Er betrachtet diese als durch Spannung verursacht und unterscheidet sie von den feineren Zähnelungen, die in einer früheren Periode bemerkt wurden. Wenn man sie aber sorgfältig beobachtet, so erscheinen sie durch dieselben Bedingungen verursacht. Darum habe ich keinen Grund, diese als amöboide Bewegungen zu erklären, wie jetzt im allgemeinen angenommen wird, sondern ich möchte die oben erwähnte Deutung vertreten. Diese Zellen, um das noch einmal zu betonen, bewegen sich dadurch, daß sie eine Oberflächenspannung erniedrigende Substanz abgeben, wie sich das Quincke (15) ursprünglich gedacht hat in seinen Studien über die Bewegung der Charazellen. Quincke hat dagegen nicht erkannt, daß die Oberflächenspannungsänderung nicht in der

Berührungsfläche von Zelle und Flüssigkeit, sondern an der Berührungsfläche der Zellen und der Proteine und Fette der Umgebung zustande kommt.

Die Wachstumsfähigkeit gehört zu den fundamentalen Eigentümlichkeiten der lebenden Substanz. Die Zellen sind befähigt, Proteine, Fette, Kohlehydrate, Salze und Wasser in sich selbst hineinzuziehen.

Sie wachsen auf Grund ihrer Fähigkeit, komplizierte organische Verbindungen abzubauen, diese zu oxydieren und dann aus diesem Prozeß Energie zu bilden. Wie die Energie freigesetzt wird und wie sie zu diesem Zwecke benutzt wird, ist nicht bekannt. Anderseits ist es sehr gut bekannt, daß Tiere wegwandern von dem Orte, wo sie mit anderen Tieren zusammengedrängt sind, zu Orten, die reicher an Nahrungsstoffen sind. Bei dem Studium dieser einfachen Zellen der Gewebekulturen war es möglich, den Mechanismus dieses Prozesses aufzuhellen und die führenden Kräfte, die dieses besondere Verhalten regeln, zu beleuchten. Die Körperzellen ziehen in sich bewegliche Proteine und Fettpartikel durch Freiwerden einer Substanz hinein, welche gleich starke Affinität für die Zelle und für Proteine und Fette hat. Wenn die Inertia dieser Proteine und Fette größer ist als die der Zellen, so werden die Zellen durch sie angezogen. Diese Bewegung der Zellen wird als Auswanderung bezeichnet.

Oberflächenspannung erniedrigende Substanz, die Ergusia, wird nicht unter allen Umständen aus den Zellen frei, sondern nur wenn eine andere Substanz, die Archusia, sich in einer gewissen Konzentration um die Zelle herum anhäuft. Die Archusia wird im Verhältnis zu dem durch die Zelle absorbierten Sauerstoff gebildet, sie wird nicht durch Körperzellen zurückgehalten, sondern wird in das Medium ausgeschieden. Sie konzentriert sich um die Zelle herum, wenn diese zusammengedrängt ist in einer gestauten Umgebung. Wenn ein Fragment von Gewebe in einen Tropfen Medium gelegt wird, so weichen die Zellen auseinander und das Fragment verbreitet sich in dem Maße, wie die Archusia sich anhäuft. Diese Verbreiterung ist die Folge des Freiwerdens einer Substanz, die die Oberflächenspannung erniedrigt zwischen den Zellen und den Proteinen und Fetten der Umgebung. Dieser Vorgang kann nicht stattfinden in einem Medium, das diese Substanzen nicht enthält. Wird die Archusia-Konzentration erhöht, so werden die Zellen befähigt, die angesammelten Substanzen zu verdauen. Wasser und Salz werden absorbiert als

die Folge dieser primären Reaktion. Das Resultat ist Wachstum. Die Zellen sind gezwungen, sich in einem Medium, welches Proteine und Fette enthält, zu verteilen, auf Grund dieses einfachen Mechanismus und nicht auf Grund irgendeines komplexen teleologischen Aktes.

In diesen Körperzellen findet man darum eine Einfachheit der Struktur, die jene der einzelligen Organismen übertrifft. In den letzten Jahren haben wir viel über Energiebildung aus dem Studium der Körperoxydation gelernt, aber sehr wenig betreffs der Methoden, die die Zelle zur Umwandlung der Energie in Arbeit benützt. Während es theoretisch sicher zu sein scheint, daß Wachstum, Zellteilung und die verschiedenen Funktionsformen sämtlich entweder Arbeit oder Arbeitsprodukt verschiedener Anteile einer äußerst komplizierten Maschine darstellen, hat bisher jeder Versuch, diese Maschine durch das Studium des einzelligen Organismus in ihre Teile zu zerlegen, nur zu Widersprüchen geführt. Ich glaube, in diesen und auch in anderen Arbeiten über Wachstum (loc. cit.) und Muskelkontraktion (16) gezeigt zu haben, daß diese Schwierigkeit überwunden werden kann durch das Studium der Körperzellen in den Gewebekulturen, weil einerseits diese Zellen in ihrem Protoplasma jeder Organisation entbehren und andererseits die äußere Haut der Amöbe und die Cutis des Paramaeciums von der Umgebung abhängt, die für einen großen Teil dieses Mechanismus verantwortlich gemacht werden kann.

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Erklärung der Tafelabbildungen.

Fig. 1. Eine Zeichnung einer schmalen Membran von epithelialen Zellen, herauswuchernd von einem schmalen Fragment der Haut des erwachsenen Frosches (Rana pipiens) in eine Schicht von Autoplasma von 0,4 mm Dicke. Die Kultur ist 14 Stunden alt. Die Randzellen und einige Seitenzellen sind abgeplattet zwischen dem Plasma und dem Deckglas. Diese flachen Zellen haben ebene Ränder. Die Kulturen sind in Formol fixiert und mit Hämatoxylin-Eosin gefärbt.

Fig. 7. Eine Photographie einer epithelialen Membran, sich von dem Rande des Fragmentes der Froschhaut bewegend, in einer 14 Stunden alten Kultur. Sie zeigt, wie sich das Gerinnsel unter dem Einflusse der Zellen verändert.

Fig. 8. Eine Photographie einer Membran von epithelialen Zellen in einem anderen Teile derselben Kultur wie Fig. 7, die zeigt, wie die Zellausläufer dieser Trennung von Fibrin und Serum des Gerinnsels entsprechen. Das Zytoplasma der Zellen haftet dem Fibrin an, welches die Wand der Serumbläschen bildet.

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A PRELIMINARY NOTE ON THE STUDY OF EXPERIMENTAL EMBRYOMATA IN MICE

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Rous states that the embryonic tissue of mice injected into host mice grows actively for only a few days or weeks and then disappears (1). In the rats conditions are different: the embryonic tissue often continues to grow for months and the tumors may then persist without change to the death of the animals. In a single experiment performed 4 years ago I noticed a teratoma in a mouse to persist for several months not unlike those then under observation in rats. As a possible contribution to the knowledge of teratomata it became of interest to ascertain the conditions under which this tumor had remained and the other had disappeared in a few weeks.

Previous authors had already noted that the growth of these experimental teratomata is prevented by the presence of cytotoxins in the serum of the host and that the relative growth of these tumors is influenced by the age of the animal (they grow better in young than in old animals) and by the amount and the age of the embryonic tissue injected. No one has studied however the relation of the exact age of young animals to the exact age of the embryonic tissue used. On the other hand it has been noticed that the onset of pregnancy, the development of a wasting disease or a malignant tumor in the host effects the growth of embryomata in rats and other animals.

METHOD

In these experiments I have attempted to control for cytotoxic effects by using close relatives of already practically homozygous strains. All of our mice had come from brother and sister crosses for 2 to 4 generations. The embryos used for injection

were always full brothers and sisters of the host. The hosts were from a previous litter by the same mother and father.

The age of the embryos was determined by noting the exact time of mating. In each case the age of the embryo is the exact time in hours from the beginning of copulation to the time the embryo was removed from the mother.

In each experiment the embryos were chopped into small pieces with scissors. A small amount of .73 per cent sterile sodium chloride was added, the mixture sucked into a syringe and injected subcutaneously into the shoulder of the host. Care was taken to see that the mass of embryonic material always remained together in one small cavity of the host's tissue.

All animals during their life were fed the same diet of grain and dog biscuits every day and carrots three times a week and meat three times a week. Abundance of water was kept in all cages.

RESULTS OF EXPERIMENTS

In the first series of experiments the equivalent of one 15-day-old embryo was injected into each host which was 42 days old. In the second experiment the equivalent of one 14-day-old embryo was injected into each host which was 45 days old. In the third experiment 13 days and 19-hour-old embryos were injected into 44-day-old hosts.

As shown in the table of Experiment No. 1, the tissue of the 15-day-old embryo grew for 11 days, when it regressed to disappear entirely after 20 days, except for small fragment of bone and cartilage. The tissue of the 14-day-old embryo grew for 15 days, when it regressed (Exp No. 2), while the 13 day and 19-hour-old tissue grew and persisted for 20 days before regression was noticed (Exp. No 3).

Experiment No. 1.

Mouse	Sex	Days of Growth 5	10	15	20	25	30	32
No 1	F							
No 2	M						–	–

Experiment No. 1. Hosts: born May 19th, weaned June 17th.

Mother impregnated again by father of hosts at 9 A.M., June 19th. 5 embryos, full brother and sisters of hosts, were removed from mother under ether at 10 A.M., July 4th. The embryos were 15 days old; the hosts were 46 days old. The embryos were chopped into small pieces with scissors. The mass of embryonic tissue was then divided into 2 equal parts. Each part was taken up in NaCl solution, .73 per cent, and injected into the shoulder of 2 hosts.

Experiment No. 2.

Mouse	Sex	Days of Growth						
		5	10	15	20	25	30	62
No. 1	F							
No 2	F							
No. 3	M							
No. 4	F							
No. 5	M							—

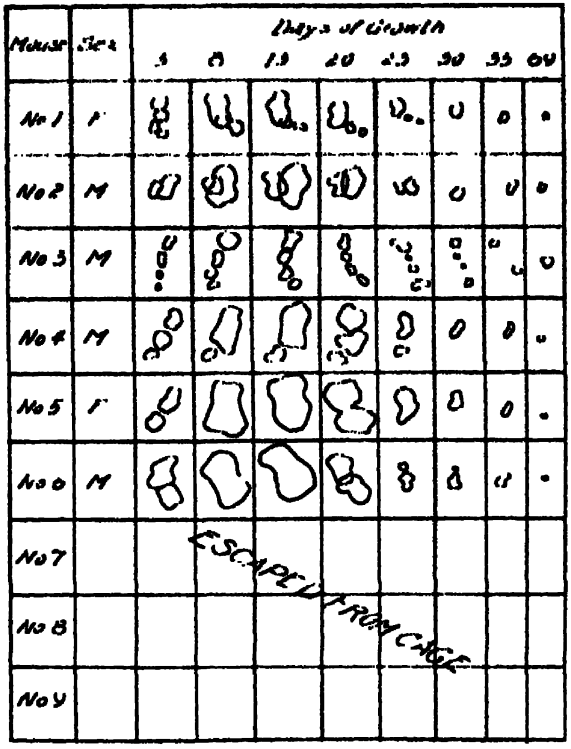
Experiment No. 2. Host: born May 20th, weaned June 17th. Mother impregnated again by father of hosts at 8 A.M., June 20th. 5 embryos, full brothers and sisters of hosts, were removed from mother under ether at 10.20 A.M., July 4th. These embryos were 14 days old; the hosts were 45 days old. The 5 embryos were chopped into small pieces. The mass was taken up in NaCl solution .73 per cent, and divided into 5 equal parts. Each part was injected into the subcutaneous tissue of the shoulder of a host.

Experiment No. 3. Hosts: born June 14th, weaned July 12th. Mother impregnated by father of hosts at 9.30 A.M., July 14th. 9 embryos, full brothers and sisters of hosts, were removed from the mother under ether at 4 P.M., July 28th. The embryos were 13 days and 19 hours old; the hosts were 44 days old. One embryo was chopped into small pieces, taken up in NaCl solution .73 per cent, and injected into the shoulder of each host.

In other experiments embryonic tissue 11 days old was injected into hosts 44 days old (Exp. No. 8): embryonic tissue 11 days and 17 hours old, into hosts 43 days old (Exps. No. 6 and 7) and embryonic tissue 13 days and 13 hours old, into hosts 51 days old (Exp. No. 9). In only two of these experiments, Nos. 6 and 9,

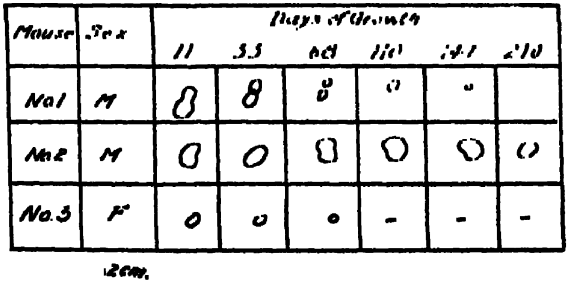
did any of the tumors continue to grow and persist for longer than 29 to 33 days. The younger embryos tended to persist longer however than the older ones. In Experiment No. 6 one

Experiment No. 3.



tumor persisted for over 150 days. In Experiment No. 9 another tumor did not regress until after 90 days. Experiment 9 differed from the earlier ones in that the tissue was injected into older hosts.

Experiment No. 6.



Experiment No. 6. Hosts: born Aug. 28th, weaned Sept. 25th.

Mother impregnated by father of hosts at 10.30 P.M., Sept. 29th. 7 embryos, full brother and sisters of host, removed from mother under ether at 3.10 P.M., Oct. 10th. The embryos were 11 days and 17 hours old; the hosts were 43 days old. 2½ embryos chopped in NaCl solution .73 per cent, were injected into each of the 3 hosts.

Experiment No. 9.

Mouse	Sex	Days of Growth 12	47	96	131
No. 1	♂	O	O	O	o
No. 2	♂	O	—	—	—
No. 3	♂	O	—	—	—
No. 4	♂	O	O	O	O

Experiment No. 9. Host born Sept. 10th, weaned Oct. 8th. Mother impregnated by father of hosts at 7.30 P.M., Oct. 17th. 7 embryos were removed from mother under ether at 8.45 A.M., Oct. 31st. The embryos were 13 days and 13 hours old; the hosts were 51 days old. One embryo was chopped into small pieces, taken up in NaCl solution .73 per cent, and injected into each of the 4 hosts.

Noting this relation of the persistence of the tumor to the age of the embryos in Experiments Nos. 1, 2 and 3 and, again, a similar relation to the age of the hosts in Experiment No. 9, it became of interest to study the growth of younger embryos in older hosts. Very few or any of the rats had reached the age of puberty at 43 to 47 days while many of them had passed this period after 48 to 50 days. Long and Evans (2) have noted that puberty in rats comes at various times. Most of our mice had reached puberty when 50 days old. The exact time has varied considerably, however, in different individuals of the colony.

In an earlier experiment, No. 4, I had injected by the same method 1⅔ chopped up embryos, 12 day and 12 hours old, in each of 5 hosts which were 53 days old. In following this experiment it was found that the tumors persisted for many months in spite of numerous pregnancies in the females. In one animal the tumor persisted throughout her life. She (No. 2) lived to be 1 year and

5 months old. The tumor was as large at her death as that shown in the table. Fig. 1 shows this tumor exposed by the removal of the skin over it after her death.

Fig. 1. An experimental embryoma in a female mouse persisting for 1 year and 3 months or to the death of the animal.

Experiment No. 4. Hosts: born June 14th, weaned July 12th. Mother impregnated by father of hosts at 9 P.M., July 24th. 7 embryos, full brothers and sisters of hosts, were removed from mother under ether at 9 A.M., Aug. 6th. The embryos were 12 days and 12 hours old; the hosts were 53 days old. The embryos were chopped into pieces in NaCl solution .73 per cent. The embryonic fragments were mixed together and divided in 5 parts. One part was injected into each of the 5 hosts.

Pieces of this tumor were sectioned and stained. It has the same structure as other tumors. It is composed of cartilage,

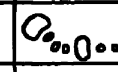
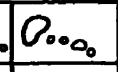
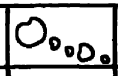
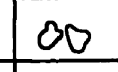
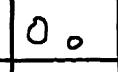
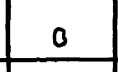
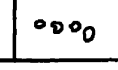
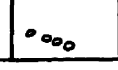
bone, connective tissue and cysts lined by skin epithelium with hair follicles, sweat and sebaceous glands and other cysts lined by a mucosa similar to that of the intestine.

Experiment No. 4.

Mouse	Sex		Days of Growth 28	49	77	108	126	212	275
No 1	M								
No 2	F								
No 3	F								—
No 4	M								lost
No 5	F			DIED IN CHILDBIRTH					

In other experiments I have continued to find tumors persisting longer in older hosts than in younger ones. In experiment No. 12 I injected embryonic tissue 13 days and 23½ hours old into rats 67 days old.

Experiment No. 12.

Mouse	Sex	Days of Growth 8	34	72	134
No 1	M				
No 2	M				lost
No 3	M				lost

Experiment No. 12. Hosts born Oct. 19th, weaned Nov. 16th. Mother impregnated by father of hosts at 6.00 P.M., Dec. 12th. 4 embryos, full brothers and sisters of hosts, were removed from mother under ether at 4.35 P.M., Dec. 25th. The embryos were 13 days and 23½ hours old; the hosts were 67 days old. Two embryos were chopped in .73 per cent, NaCl solution and injected into host number 1. Only one embryo was injected in the same manner into hosts, Nos. 2 and 3.

CONCLUSION

From these observations it is evident therefore that teratomata produced by injecting chopped-up mouse embryos into host mice may persist under the proper conditions and there seems to be a definite relation between the age of the embryo and the age of the host to this persistence. The younger embryonic tissue persists longer in the hosts than that of older embryos. Hosts which have just passed puberty are more favorable for the persistence of the tumors than younger ones. One animal inoculated with $1\frac{3}{4}$ chopped-up embryos when it was 53 days old carried the tumor throughout its life in spite of several pregnancies.

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PROCEEDINGS OF THE WASHINGTON UNIVERSITY MEDICAL SOCIETY

One Hundred and Tenth Meeting, February 9, 1925

2. A STUDY OF CELL STIMULATION, CANCER AND VITAMINS.—By Drs. Montrose T. Burrows and Louis H. Jorstad.

The conditions allowing a growth of body cells in the tissue culture have been studied. In a simple normal body fluid such as the plasma of the blood of a normal individual single cells cannot grow. Growth can intervene only where many cells are crowded together in a small amount of stagnant medium so that a normal product of their oxidation reaction can be made to remain about them and accumulate to a certain concentration. This substance formed by all cells cannot be retained by the cell. It is readily washed away by circulating blood. This substance has been called the *archusia*. Cells can be made to grow by adding this *archusia* to the medium as by allowing it to accumulate from the cells, which are to be made later to grow by it. *Archusia* extracted from other stagnant cell masses is as effective as that formed by the cell itself. The independent growth in cancer is the result of the fact that cancerous tissue is a dense mass of cells poor in blood vessels. It is a tissue which can form a large quantity of *archusia* on account of its densely cellular content and this remains because of its poor circulation or no ready means for it to escape.

In applying these laws to the normal organism it was noticed that many tissues even in late embryonic life have too active a circulation and too few cells per unit capillary area for them to grow. The growth of these tissues must obtain, therefore, through the blood obtaining *archusia* for them from other sources. We have sought a supply of this *archusia* in the food and in the glands of internal secretion. We have found that many non-pathogenic as well as pathogenic bacteria form an *archusia* which acts readily to stimulate the cells of the body to grow. When these bacteria are fed the body grows actively. They act as vitamin B. The organism with its active circulation undoubtedly survives not because of an ability for its cells to grow independently, such is possible only in cancer, but by preying on lower growing forms for a part of their growth energy. The bacteria particularly studied were the B. tumefaciens and B. campestris. See also article by Burrows, M.T., 1925, Proc. Soc. Exp. Biol. Med., Vol. XXII, p. 241.

3. THE OVARIAN HORMONE AS A CELL STIMULANT.—By Drs. Montrose T. Burrows and Charles G. Johnston.

In this paper we have studied the action of the ovary as a stimulus for the growth of body cells. The extracts containing *orchasin* stimulate the cells of the body to digest fats and proteins and grow. We find that mazola oil injected under the skin of rats remains as such for an indefinite period. It is not used by the cells under normal conditions. In other experiments the Allen Doisy Hormone was added to the oil injected. The cells invade these oil droplets, digest them and grow with great activity until all the oil has been removed. It has been known for a long time that the ovary has a definite effect on the fat metabolism of the organism. By these experiments it has been not only possible to give direct evidence for the manner in which it acts in this capacity, but to demonstrate also a source of growth stimulus in this internal secreting gland.

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AN EXPERIMENTAL STUDY OF THE RELATION OF THE OVARY TO FAT METABOLISM.

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PLATES 4 TO 6.

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The controlling influence of the ovary on cyclic changes in the uterus and breasts has been fully appreciated for many years. Recently Allen and Doisy (1) have isolated from the follicular fluid of the ovaries of pigs an alcohol-, ether-, and acetone-soluble substance, which induces estrus when introduced subcutaneously into spayed rats.

It is also known that the ovary has to do with the metabolism of fat in the body. Women store fat after the removal of the ovaries, destruction of the ovaries from one of various causes, and after the menopause. Women suffering a premature loss of function of their ovaries also suffer various other metabolic changes and disturbances. In order to throw light on this latter property of the ovary it became of interest to study the action of the Allen-Doisy hormone in the digestion of fat in the tissues.[1]

In a previous study (2) we have shown that Mazola, or corn oil, which had been sterilized by heat, is not absorbed when injected into the subcutaneous tissue of most rats. In only one case out of thirty-nine was there any evidence of its absorption even after periods as great as 7 to 9 months (Fig. 3). This oil in the great majority of these cases simply breaks up into numerous small and larger droplets. Each of these droplets then becomes encapsulated by cells.

In this previous study we also described the method of the encapsulation of the oil droplets. These capsules are formed chiefly by large spherical shaped cells, having a single poorly staining nucleus. These cells are closely packed together at the edge of the oil droplet, forming a capsule one to several cells in thickness. Among these large cells are a few lymphocytes and neutrophils, and eosinophils, and polymorphonuclear and mononuclear leucocytes. The number of these

[1] Read before the Society of Experimental Pathology, Washington, D. C., December 29, 1924.

latter cells is never large and is the number that may be present at any time in blood vessels and tissue adjacent to the place where the oil was introduced.

This capsule is formed primarily of cells which migrate to the oil from the surrounding tissues. The large round cells are fibroblasts and endothelial cells, which have been drawn with the lymphocytes and leucocytes to the oil from the surrounding connective tissue and capillaries. None of the cells of the capsules have formed through proliferation. They have migrated to the edge of the droplets of oil from the tissue. During this migration the fixed tissue cells have not only rounded off to spherical shaped cells, but have suffered a loss in their ability to stain sharply.

This process of migration ceases after 48 hours. Subsequent to this time the fibroblasts and endothelial cells gradually regain their ability to stain sharply. They lay down intercellular fibrils, stretch out along the surface of these fibrils, and assume spindle shapes. In a few cases where large numbers of these cells had become crowded together at the edge of the droplets, the nuclei of the cells show an increase in chromatin. One division figure was seen in one specimen of this kind during this later recovery period. As a rule, however, the only evidence of growth is the formation of intercellular fibrils. Subsequently these cellular fibrous capsules then slowly regress to a hyaline scar (Fig. 3). The leucocytes and lymphocytes remain unchanged for a time in the capsule. Then they gradually disappear.

Corn oil does not stimulate a growth of cells. It acts to cause regressive rather than constructive changes in them. Evidences of growth about these oil droplets manifest themselves late, after the oil has ceased to attract the cells from the surrounding tissue and otherwise act upon them. This recuperation of the cells and growth are more and the amount of earlier degenerative changes in the cells is less about the smaller droplets than the larger ones.

Allen and Doisy had dissolved their hormone in corn oil in order to facilitate its introduction into the subcutaneous tissue. In each instance a tumor formed at the site of the injection. Since oil produces such tumors it had been thought that this reaction was due alone to the oil. In order to throw light on the mechanism of the absorption of the corn oil in one of our animals and not in the others, and in view of the relation of the ovary to fat metabolism in the organism, it became of interest to remove and study histologically a number of these tumors produced by the oil plus the ovarian hormone.

The animals used for these experiments were 60 rats, 2 guinea pigs, and a monkey. The hormone was prepared by first extracting the fresh follicular fluid

from the ovaries of pigs with alcohol, filtering, and evaporating the alcohol solution to dryness. The residue was then extracted with ether. After the ether solution was filtered it was evaporated and the residue extracted with the corn oil. This oil-hormone mixture was then introduced into the subcutaneous tissue of the animals.

The corn oil containing the hormone when introduced into the tissue breaks up into droplets the same as the pure corn oil. It excites likewise a rapid migration of cells to it from the surrounding tissues. A capsule forms which is identical with that seen about the pure oil except that the cells in migrating to the oil show very little evidence of degeneration, but rather an early active growth and division and an early and active laying down of intercellular fibrils. The cells also invade the oil, remove it, and proliferate most actively in the space occupied by it. Many of the smaller droplets of oil are completely removed by this process and the space originally occupied by them becomes a dense mass of proliferating fibroblasts. In the larger droplets this process ceases generally after a small amount of the oil is removed. After a given period of such proliferation this mass of new cells then slowly regresses and disappears in the form of a hyaline scar.

The details of this process are illustrated in the description of experiments below.

Experiment 1.—Two guinea pigs were injected with 1 cc. of corn oil containing the ovarian extract. The tumors were removed after 6 days. These tumors, like the tumors formed by injecting pure corn oil, are composed of numerous encapsulated droplets of the oil. Each of these droplets is surrounded by a layer of cuboidal cells. Outside this layer are layers of fibroblasts which at this stage have laid down a considerable amount of intercellular material and have stretched out to a spindle shape. Mitoses are numerous not only in the layer of cells about the oil droplets (Fig. 1), but also in the layer of cells deeper in the capsule. In many places these inner capsular cells are proliferating rapidly, the oil is disappearing, and the space occupied by it is gradually being replaced by these proliferating cells (Fig. 2).

As shown by the later experiments this proliferation into the smaller droplets continues until the oil is entirely removed and its site replaced by a dense mass of proliferating cells.

Experiment 2.—A rat was injected with 2 cc. of Mazola oil containing ovarian extract. The tumor was removed after 3 weeks. About all of the oil cysts the cells are seen proliferating actively. Many of the smaller drops of oil have been

entirely removed and replaced by these growing cells (Fig. 4, *A*). In other cases the cells are seen to be growing and slowly invading the oil from all sides (Fig. 4, *B*). The larger droplets of oil are still present (Fig. 4, *C*). Their former single layer of lining cells is seen now, however, as a layer many cells in thickness. About the edge of these larger oil droplets cell processes can also be seen projecting out into the oil as they are seen in Fig. 4, *B*.

Experiment 3.—59 rats were injected with 1 to 5 cc. of corn oil plus the ovarian extract. The tumors were removed from time to time, between 1 week and 7 months thereafter. The sections of tissue removed within 1 month show pictures similar to that seen in the guinea pigs and rat of Experiments 1 and 2. Subsequent to this time the proliferating tissue slowly regresses. In the great majority of the cases only the smaller oil droplets are found to have been removed by the cells. In other sections all of the oil has been removed. Only the original fat of the subcutaneous tissue remains. This active proliferation of cells continues, however, for only about 2 to 3 weeks, when it ceases and regression takes place. The denser masses of cells which fill many of the small oil cavities lose their nuclei. Their cytoplasm unites with that of neighboring cells. It becomes more granular and stains less sharply (Fig. 5). In the lower power picture of these areas after 6 weeks to several months one finds a few oil drops remaining and other open spaces which resemble the lacunæ left by cholesterol crystals (Fig. 6). Finally this mass of cells which filled the oil space shrinks to appear as a single large cell containing no nucleus or one or more poorly staining nuclei. The surrounding tissue undergoes slow regression to a hyaline scar containing a few small oil droplets. This process of regression takes place more quickly in some animals than in others.

Experiment 4.— One spayed monkey received 1 cc. of the corn oil plus ovarian extract. The tumor was removed after 3 months. The oil has been completely removed from this tumor. The tumor is composed of a cellular mass regressing to a hyaline scar (Fig. 7). The open spaces shown in this figure are for the most part the fat droplets of the original tissue or secondary fat deposits in the regressing scar.

DISCUSSION AND CONCLUSIONS.

From these observations there seemed to be little doubt that the follicular fluid of the ovary contains an active growth-stimulating substance and one capable of initiating an active digestion of a foreign fat, which might otherwise remain unabsorbed for an indefinite time in the tissues of these animals (2).

We have not attempted to ascertain whether this substance exciting growth and a digestion of the oil is the same or in any way related to the substance exciting estrus in these animals. That it may be a different substance from the estrus-exciting substance is suggested,

however, by the fact that a similar excitant of growth and fat digestion has recently been extracted by the same method from the corpus luteum of pigs. These extracts of corpora lutea have not excited estrus in spayed rats.

In the one rat in which the pure oil was absorbed, the cells did not invade the oil, but the capsule remained cellular and the oil gradually disappeared from the space. In these experiments in which the active substance was added to the oil the cells have always invaded the oil.

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EXPLANATION OF PLATES.

PLATE 4.

FIG. 1. A moderately high power photomicrograph of a part of a section of a 6 day old tumor of a guinea pig formed by injecting corn oil plus ovarian hormone.

FIG. 2. A moderately high power photomicrograph of another part of the section shown in Fig. 1.

PLATE 5.

FIG. 3. A high power photomicrograph of a part of a section of an 8 months old tumor of a rat produced by the injection of 6 cc. of pure corn oil into the subcutaneous tissue. This picture is included to facilitate comparison between the action of pure oil and oil plus ovarian hormone.

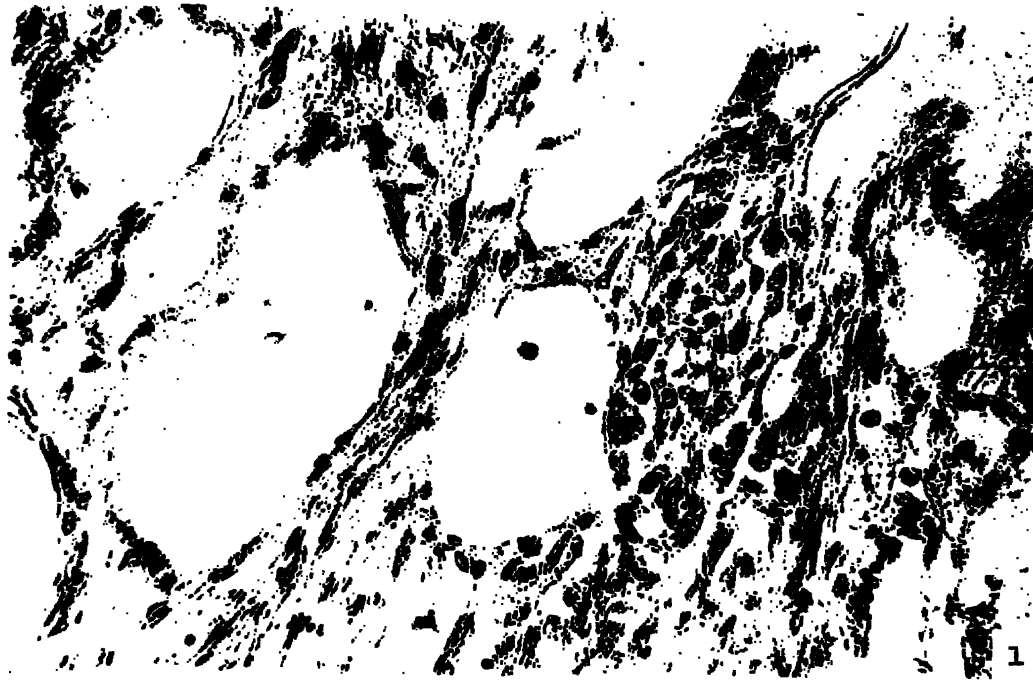
FIG. 4. A moderately high power photomicrograph of a part of a 3 weeks old tumor of a rat produced by injecting corn oil plus ovarian hormone.

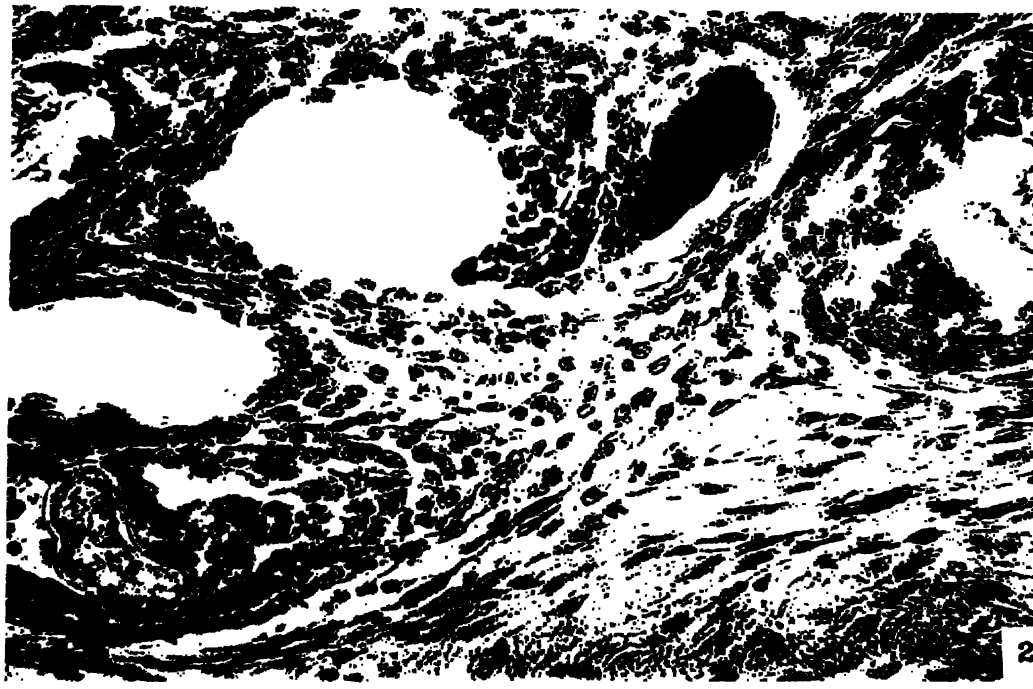
PLATE 6.

FIG. 5. A moderately high power photomicrograph of a section of a 7 months old tumor of a rat produced by injecting corn oil and ovarian hormone.

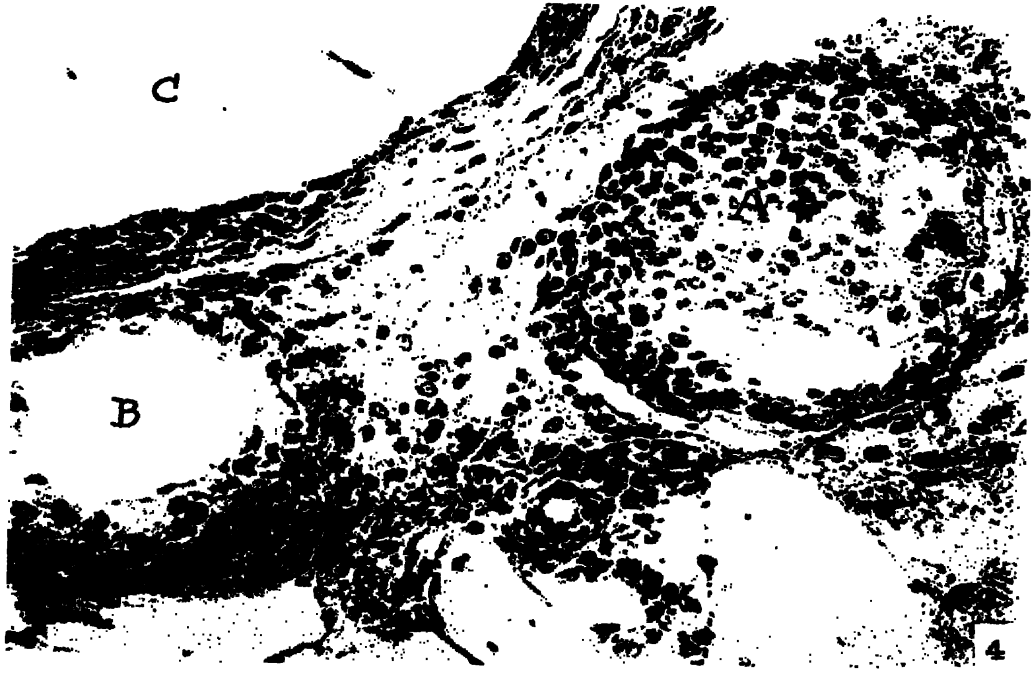
FIG. 6. A low power photomicrograph of the section shown in Fig. 5.

FIG. 7. A high power photomicrograph of a 3 months old tumor of a monkey produced by injecting 3 cc. of corn oil plus ovarian hormone.





(Burrows and Johnston: Relation of ovary to fat metabolism.)



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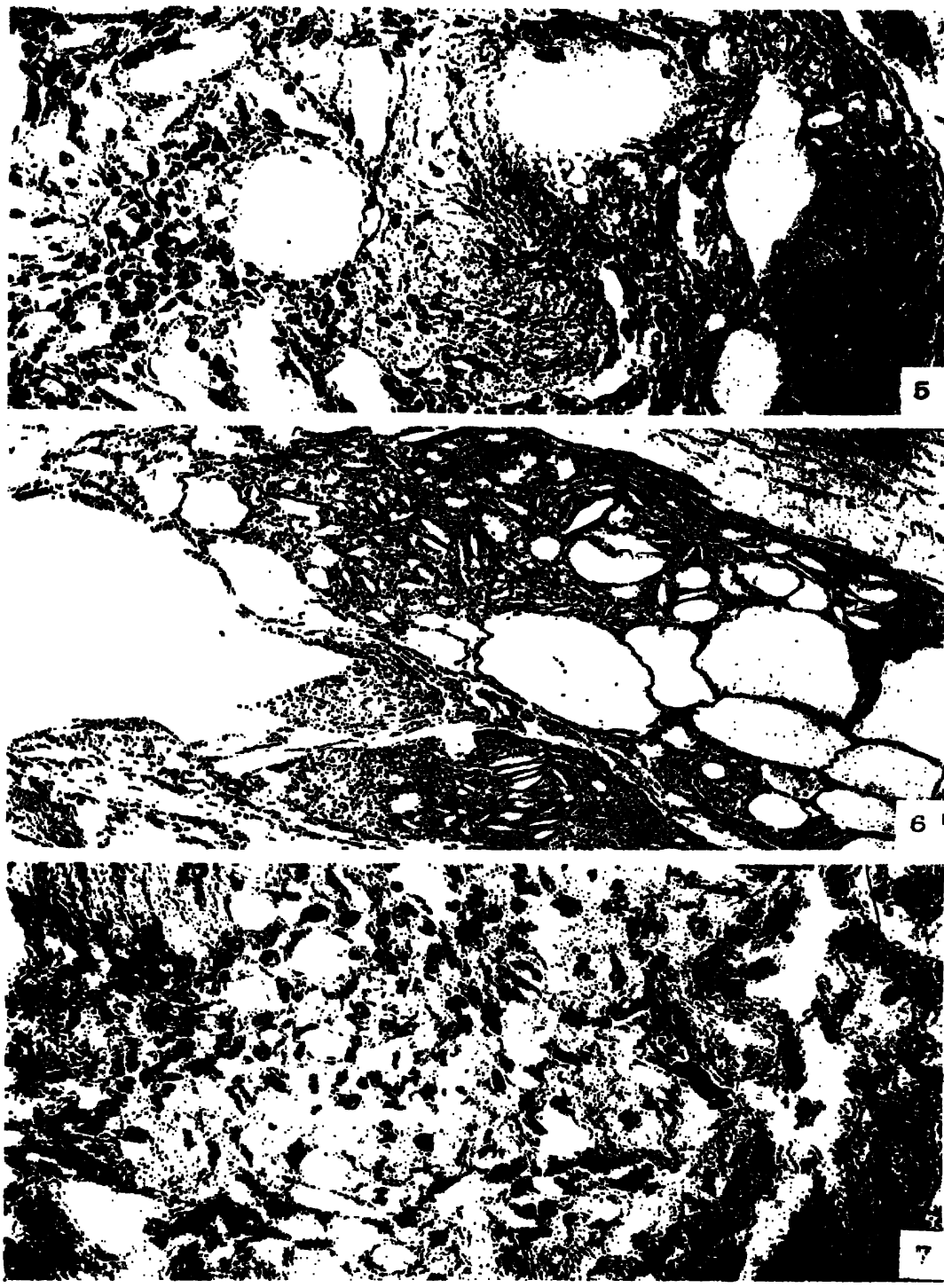

(Burrows and Johnston: Relation of ovary to fat metabolism.)

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THE BEHAVIOR OF COAL TAR IN ADULT AND EMBRYONIC TISSUE

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While many authors since as early as 1906 have noted that any one of various lipoid solvents will induce growth in body cells when injected locally, no one succeeded in producing carcinoma by this method until Yamagiwa and Itchikawa (1) had completed their work in 1918. The earlier authors had studied the effect of single applications or injections. B. Fischer (2) in 1906 introduced a drop of olive oil saturated with Scharlach R under the epidermis of the skin of the ears of rabbits. The adjacent epithelial cells of the epidermis and hair follicles grew down to enclose the oil droplets and to produce a picture not unlike that seen in cancer of the skin. The growth stopped in these cases after a short time when the oil was absorbed. Reinke (3) 1907 noted atypical growths of epithelium after the injection of a 4 per cent solution of ether into the eye of a salamander. Pieces of these growths transplanted into the peritoneum of other salamanders continued to grow for a time. Many of them resembled carcinomata in many of their morphological characteristics. Askanazy (4) added ether to emulsions of embryonic tissue. He found these emulsions when injected into hosts grew more vigorously than those which had not been treated with ether.

So in the same manner many workers showed that any number of lipoid solvents produce atypical growths but none of them produce cancer (Wacker and Schmincke, Bullock, Rohdenburg and others) (5). It remained then for Yamagiwa to appreciate that cancer develops in the skin of coal tar workers and chimney sweeps only after they have been contaminated with these

substances for a very long time. While Yamagiwa and Itchikawa found atypical proliferation after a single inoculation into rats, true tumors of a benign and malignant character did not develop until after they had repeatedly applied coal tar to the same area of the skin for a long time. These authors painted tar on the skin of the ears of rabbits. The results of their experiments are essentially as follows: (*a*) papillomatous new growths, termed folliculo-epitheliomata, may be produced on the rabbit's ear by the application of coal tar for 30 to 100 days; (*b*) by the repeated application of coal tar, 8 cases of carcinomata in the earliest stages, 16 in an early stage, and 7 complete carcinomata were produced. The carcinomatous change was discovered between the 35th and 360th day; in most cases it was found after the 150th day; (*c*) the hyperkeratotic pedunculated or sessile folliculo-epitheliomata produced by irritation with coal tar continued to grow after the irritant had been discontinued, and eventually developed into cutaneous horns. Some of these horns grew a year after the withdrawal of the coal tar, while others fell off spontaneously. In many of the latter animals from which these horns primarily disappeared, new cutaneous horns grew again from the same base or from the neighboring epithelium as is the case in man; (*d*) their seventh and sixteenth case of early carcinoma developed from cutaneous horns about 300 days after the tar had been discontinued; (*e*) the presence of metastases was microscopically proven in the regional lymph nodes in their fourth and sixth cases of carcinomata; (*f*) the animals which bore folliculo-epitheliomata did not begin to emaciate while the new growth maintained its benign character.

By these observations it was possible, therefore, for Yamagiwa to introduce the first important point in relation to the development of cancer in the skin of certain tradespeople. It is not single but repeated exposures to the irritant which count in these cases. Since that time many experiments have been made with the object of not only confirming these earlier results but of ascertaining the action of these lipoid solvents. Tsutsui (6) has produced carcinoma on the skin on backs of mice by painting with coal tar. His method was the same as that used by

Yamagiwa on the ears of rabbits. He observed 16 carcinomata and one sarcoma in 17 mice surviving over 100 days. In 2 cases lung metastases were found. He also found one fibro-myxo-sarcoma after coal tar had been injected into the mamma of rabbits over two years time. By using Sudan III in olive oil, drop at a time, at 5 or 6 day intervals repeated 17 times in two rats which died on 42d and 57th day, he transformed a benign tumor of white rats into a malignant one. He transplanted this malignant tumor successfully through 14 generations.

Lipschütz (7) reports that his efforts to produce carcinomata in mice by means of external application of coal tar yielded positive and tangible results in about 45 per cent of the trials. At least inflammations of the skin were observed frequently; necrosis, however, was not noted in a single instance. As to the time that elapsed before the first skin changes became evident, he states that one animal showed the first distinct microscopic changes in 88 days, two in 112, and one in 25 days. These changes were wart-like formations which persisted for many weeks. In two instances, subcutaneous transplantations of these wart-like nodules to healthy mice resulted in the development after 19 days of skin changes characteristic of coal tar. These old warts induced wart-like growths, although the transplanted tissue itself was completely absorbed. One tumor arising from these transplants when incised, proved to have a coarse-fibrous structure throughout which resembled sarcoma in many ways.

Deelman (8) reports researches begun in 1917. He painted the ears of rabbits and the backs of white mice with coal tar three times a week. 25 illustrations show the findings. In 26 of the 48 mice a carcinomatous ulcer developed and in the others a papilloma which resembled the first stage of cancer. These tar cancers resemble human cancer much more closely than spontaneous tumors in animals. The malignant growth that developed in one of the animals proved to be a spindle cell sarcoma with metastasis in the lung and pleura, but not in the lymph glands. This tumor was transplanted into other mice and has grown through eleven generations to date. The trans-

planted grafts grew rapidly and became large in 6 or 7 weeks. The successful inoculations were 4 out of 5; 2 out of 6; 11 out of 21; and 7 out of 10 in the first 8 generations. Transplanted pieces of the first generations of carcinomata which he developed did not "take." The tissue melted away in the abscesses that formed. This was probably due to the fact that all of the carcinomata were ulcerated from which the grafts were taken. The tar from the gas works where horizontal retorts were used seemed to be more active than tar from vertical retorts.

Murray and Woglom (9) studied the action of tar painted on the back of mice. They found a malignant metastasizing carcinoma developing in a few of their mice. They emphasize the fact that the carcinoma developed in these animals when they had reached a period of their total life cycle which corresponds to about the 35th year in man.

Fibiger (10) gives a historical sketch of the cancers that have been found in man from irritation with soot, pitch, etc. He begins with Pott's description in 1775 and brings the work to date. He also reviews his own work with cancers induced in mice by painting the back with coal tar. He has been more constantly successful so far than others—fully 24 of his 26 mice have developed papillomata. One carcinoma was transplanted through 4 generations in four months with "takes" in from one to six animals in each generation. A recent communication from Fibiger reports metastases in 22 of 85 mice with carcinomata or sarcomata. Sudorff working in Fibiger's institute at Copenhagen, has succeeded in inducing an adenocarcinoma in the mammary gland of a mouse by injecting minute amounts of tar into the breast over a long time. This is the first experimental mammary adenocarcinoma to be produced by this method. Fibiger remarks that these tar cancers put the finishing touch to Virchow's theory of the casual importance of irritation in cancer, but the fact that cancers do not invariably develop proves that a predisposition may be indispensable. He also notes evidences of an organ predisposition.

Fibiger cites about 15 research workers who had been striving between 1889 and 1916 to induce malignant tumors by repeated

applications of pitch, tar, aniline, etc. All were on the right track, but none repeated the application of the tar long enough until the Japanese finished their work in 1918. He also notes that rats and mice are probably more susceptible than rabbits. He and Bang (11) obtained positive results in 90 per cent of the white mice which survived over 3 months after the initial application of the tar, while Yamagiwa and Itchikawa obtained only 12 malignant tumors in 200 rabbits. Tsutsui succeeded in producing tumors in 50 per cent of his experimental mice and Bierich (12) in 60 per cent.

Subsequent to these earlier experiments, the relations of these tumors to true cancer in man and lower animals and the action of coal tar and other lipoid solvents became important questions for discussion in the literature. The first of these problems needs not be discussed here. There seems little doubt at the present time that the earlier criticisms of Bullock and Rhodenburg (1915) have been answered. True cancer can be produced not only by repeated applications of coal tar but also by any number of other substances and conditions. How the coal tar and these other substances act remains as yet a debated problem. It was this latter question that interested us particularly.

In looking over the literature of this phase of the general problem it has been interesting to note that many authors have considered cancer to be a disease like typhoid fever, a reaction to a specific entity. This is seen in the work of Nuzum (13) and Ochsner (14). Bullock and Rhodenburg, Lipschütz and others have thought that irritation alone is not sufficient in determining cancer. Leitch (15) found that when tar is applied repeatedly for a certain length of time and the irritant is then removed, tumors, even carcinomata, may make their appearance at a later date. He thought that irritation produces some profound change in normal cells subjected to its influence which is undetectable by the microscope but which allows them to proliferate eventually in an unrestrained and harmful fashion. He believes that it may be concluded that the neoplastic response to an irritant is a slow tissue reaction that exhibits no defensive property and that subserves no useful function. The internal changes in the cells in the earlier stages of irritation are unknown.

J. A. Murray (16) conducted experiments in which unaltered tar, alcoholic extract, and ethereal extract of tar were applied to separate areas of the dorsal skin of sixty normal mice. After four months, when the first tumor appeared, fifty mice survived, and of these, 25 presented malignant new growths. Twenty-two animals bore carcinomata at the site painted with the ethereal extract: 12 had carcinoma at the site treated with the original tar, and two only had carcinomata at the site treated with the alcoholic extract. In one of these animals no tumors appeared at the sites treated with the apparently much more efficacious whole tar and ether extract. Mook and Wander (17) have found that camphorated oil (paraffin oil) does not produce cancer but scar-like formations in adult men, when introduced under the skin. These tumors appear from 2 to 18 months after the injection. They are composed of droplets of the oil surrounded by tough hyaline and fibrous capsules. The reaction to this oil in the subcutaneous tissue of man resembles the reaction to other foreign bodies.

C. S. Engel (18) emphasizes the fact that the chemical irritants inducing cancer have an affinity for lipoids, and therefore for nerve substance. Several authors have pointed out that a connection may exist between the nervous system and the development of cancer. After a study of intestinal carcinoma in *Schistomiasis japonica*, Kazama, (19) holding to this latter idea, has been trying since 1919 to produce tumor formation on the mucous surfaces of viscera by the action of various stimuli. He has succeeded in producing adenomatous cancer in the gall-bladder of guinea pigs. When the surface of the mucous membrane of the stomach, urinary bladder, or gall-bladder of the rabbit or guinea pig is irritated by either mechanical or chemical means for a certain period of time polypous, papillous or adenomatous growths or even adenoma or adenocarcinoma form at the places irritated. The new growths have also been seen to metastasize.

Many of the recent English school led undoubtedly by the earlier idea of Ross (20) have looked for some specific substance in coal tar which acts on the cells to change them and stimulate

their growth. Most of these authors have considered cancer to be the result of a primary change in the cell. Why these authors have taken this view I do not know. It is not coal tar alone which produces cancer. It is any one of a large number of different substances and conditions such as many lipoid solvents, X-ray, radium, arsenic, chronic inflammation, heredity, etc. As Burrows (21), (22), (23), (24), (25) has already pointed out clearly, cancer is not a reaction to a specific entity like typhoid fever. It resembles gangrene in that it may arise after the action of any number of conditions and substances. The important problem in cancer is the nature of the conditions which regulate the normal growth of cells in the organism. The problem of gangrene was solved by the discovery of the necessity of a proper blood supply to tissue for them to remain intact, so cancer will be solved by an understanding of the conditions which regulate normal growth in the organism and not by any minute chemical analysis of coal tar. There is no evidence that the normal cell cannot grow under proper conditions of its environment. The various conditions which produce cancer cannot be classified by any chemical peculiarities. Their common action must be physical.

Not only on account of the differences of opinion and results held and obtained by the authors but also on account of recent experiments carried on by Burrows here in the laboratory he asked me to undertake a study of the action of coal tar. I have used white rats for these experiments and have studied the effects of single and multiple injections of coal tar on various types of tissue structures such as the mesenchyme in the embryo and the connective tissue in the adult and the epidermis of adults and embryos.

Burrows has shown that when tissue cells are placed in a drop of plasma the cells invade the medium. This invasion is the result of a specific adsorption or chemical affinity of the fibrinogen for an "L" substance, (*ergusia*) formed in the cells. The migration of cells in plasma clots is purely the result of a specific adsorption of the *ergusia* by the fibrinogen. This *ergusia* is also adsorbed by fats and probably carbohydrates, but not by water.

It is not liberated under all conditions by body cells. Isolated body cells cannot liberate this substance except when they are either just removed from a stagnant environment or when the environment about them fails to absorb and dilute products of their oxidative reactions. The formation of the *ergusia* depends primarily on the accumulation of a certain other product formed in the cell's oxidation, the *archusia*. In this substance the energy for all of the reactions of the cell is found. In low concentration the *archusia* has no effect In the next higher concentration it leads to the liberation of the *ergusia*. Mobile proteins and fats are drawn into the cell as the *ergusia* is adsorbed by them. Fixed masses of proteins and fats draw the cells to them. In the next higher concentration of the *archusia* the proteins and fats are digested—the cell grows. In still higher concentrations the cell itself is digested. Where body cells differ from the lower organisms is that they have lost the power either to retain the *archusia* or to form it under all conditions. To collect about them the proper concentration of *archusia* for growth they must be crowded together. An active circulation also prevents the accumulation of the *archusia*.

Applying these facts to development, Burrows notes that cellular growth in the embryo slows with the development of the blood vascular system and the separation of cells. The cells which dominate in the healing of clean skin wounds are the epithelial cells which are massed together and are free from an intercellular capillary net. In a further study of tissue cultures and healing wounds he has shown that any cellular mass of tissue having a reduced circulation may dominate over and eat up a less cellular mass no matter what may be the type of the cell in either of the two. He has found no evidence indicating that the cancer cell is different from a normal cell. The active growth in cancer, its tendency to digest itself and its ability to prey or feed on the remainder of the body is directly the result of the fact that it is a cellular tissue having a reduced blood supply. Its increased energy depends entirely on this fact.

The cause of cancer as Burrows has shown is the direct result of any substance or conditions which primarily crowds any type of cells together and reduces the blood supply to the mass.

As I have stated above, Burrows has shown that the migration of body cells is not like that of the amoeba or the paramoecium. It is the direct result of the liberation by them of a substance which decreases their surface tension in the presence of proteins and fats. Mobile proteins and fats are drawn into the cell. Fixed proteins and fats draw the cells to them. Coal tar is a viscid fluid which tends to remain fixed in the tissues. In my experiments I have found no evidence to show, therefore, that coal tar acts other than to adsorb the *ergusia* and thus draw the cells to it. It thus acts to produce a cellular growing mass under one set of conditions or a hyaline scar under another. This difference is related to the original cellular and other characters of the tissue and the technical peculiarities of the experiment. Where one draws a large number of cells about the tar and the cells are not destroyed by a too great loss of lipoid from them, growth intervenes. Where smaller numbers are drawn about the drops—hyalinization eventually develops. Where the amount of *ergusia* taken from the cells is more than is forming, they degenerate.

EXPERIMENTAL DATA

Injection of Coal Tar into the Subcutaneous Tissue of Rats

In the first 12 male and 10 female rats one cc. of coal tar was injected subcutaneously at the same place every seventh day. After the third injection the rats showed definite symptoms of malaise; they lost weight; their coats became yellow; and their hair ruffled. The cages were cleaned at this time and the injections were stopped for ten days. Tumors developed in all of these rats. At different periods throughout the experiment certain of the animals were killed and autopsied: sections were made from the tumors, and a study was made of internal organs of the animals. Sections taken from a specimen four days following the first injection of coal tar shows a definite encapsulation of the product by a dense mass of fibroblasts. In many of these tumors the cells show early degenerative changes. Beyond this dense mass is a fibrous capsule with scattered fibroblasts (Fig. 1). Section taken eighteen days after the initial injection

shows a few fibroblasts about the coal tar and evidence of hyalinization of the fibrous tissue about the drop. Not only the fibroblasts but the connective tissue fibrils are degenerating. The tumor removed at this time was 5 mm. in diameter. Microscopically it looked like the encapsulation of any foreign body with a degeneration of the capsule superimposed (Fig. 2).

Thirty-five days after the initial injection, the injections were discontinued in two of the animals and it was noted that the tumors disappeared gradually and on the 28th day following had been completely absorbed. After this time, the injections were continued again. The third and following injections were increased to one cc. but no tumors were produced after four months time at the point where the earlier tumors had disappeared. One month later these animals were killed and autopsied. They had no tumors. Even the coal tar had disappeared from the site of the injection.

In the remaining animals injections of the coal tar were continued at 10 day intervals. A mass persisted and gradually increased in size at the point of injection. Three months after the initial injection one animal was killed and autopsied. The general autopsy was negative, section of the tumor (average size in the series) showed an area of granular degeneration immediately about the coal tar. Outside this layer there were large round cells and granular detritus. These large round cells resembled connective tissue cells which had changed their shape. Beyond this zone a fibrous and partially hyalinized capsule existed (Fig. 3).

Injections were continued in other of these animals at fifteen to thirty day intervals for a period of 4 months longer. Autopsies were made at the end of the fourth month. The different tumors showed different kinds of changes. Many of the masses of coal tar were surrounded by a dense hyaline, degenerated fibrous capsule in which there were no cells. In one tumor the picture changed. The coal tar was encapsulated by a mass of fibroblasts 20 to 40 cells thick. Beyond this there is a cellular fibrous tissue. Aside from shriveling of the connective tissue near the coal tar and a greater number of intercellular fibers the

capsule is much like that seen about the larva of the cat tapeworm in the liver of rats which as Bullock and Curtis (26) have shown, frequently becomes sarcomatous.

The degeneration in these cells is striking in that it appears as a shriveling of the whole cell and then a fragmentation of the nuclear material of the cells. The broken up nuclear material stains sharply with hematoxylin.

Two of the animals of this first series were living at the end of 11 months, at which time they were autopsied. No metastatic tumors were found in either case and the tumor mass was composed of hyaline fibrous tissue.

In a number of these animals I made only a single injection of coal tar. This coal tar with the original mass of tissue, was removed after 2 to 30 days and sectioned. In these cases the picture was the same. The fibers of the original connective tissue were pushed back by the coal tar to form a capsule. Into these fibers the cells had migrated from the surrounding tissue. These were always few in number and scattered. Near the coal tar the cells showed shriveling or degeneration in the earlier periods. Later this capsule underwent hyaline change. Even in the earlier stages the endothelial cells lining capillaries and even larger blood vessels often disappeared as such in the region about the coal tar (Fig. 8).

When the tar is injected into the derm just beneath the epidermis another phenomenon is noted and that is a shrinkage of the tissue about the drops of tar. This is well shown in Fig. 9. This specimen was removed 180 days after the injection of a few drops of tar. In the region of the drops of tar the connective tissue has been drawn together. The fibroblasts have increased slightly and the epithelial layer has thickened and there are papillary extensions from this layer along the needle tracts. At this late stage the process has stopped, however; the horny layer in the thickened part of the epithelial layer has thickened so that the Malpighian layer is little thicker than that of the surrounding skin (Fig. 9).

Only in a few of these cases where the tar was introduced repeatedly into the same place over a long time did I note any

evidence of proliferation in the cells. I am not certain that any actual growth of connective-tissue cells took place even in most of these regions. About only a few of the tumors did the connective tissue become very cellular. In the epithelial layer conditions are different. In this more cellular layer growth follows even the earlier extensions of epithelial cells towards the tar unless these cells are destroyed by contact with the tar itself.

A Study of the Changes Produced by Single and Repeated Injections of Coal Tar into a Series of Experimental Embryomata of the White Rat

In a series of fifty-four white rats, embryomata were produced in similar hosts in the following manner: A female white rat was impregnated at a stated time, the resultant embryos were removed at a stated time, the interval denoting the age of the embryos in the particular animal. The embryos were chopped into small pieces with scissors and the pieces in salt solution were injected subcutaneously into the axillary region of young rats. The hosts were previous offsprings of the parents of the embryos. In each experiment two chopped-up embryos were introduced into each host. The reaction of the salt solution used was 7.4 Ph.

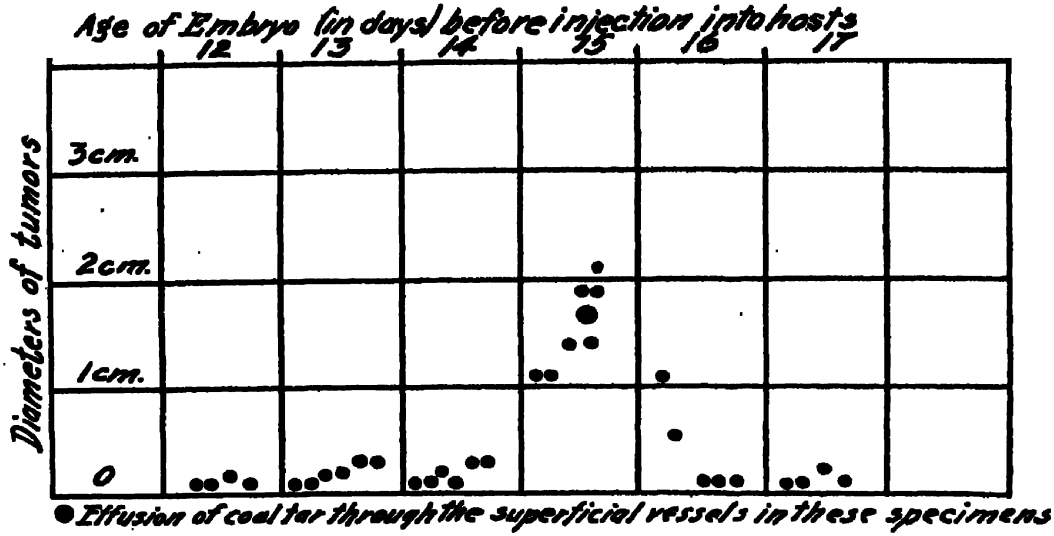
It was found that thirty days' old embryomata produced by injecting chopped-up 15 day old embryos were the best for the study of changes brought about by the repeated injections of coal-tar. This I determined by injecting different dilutions of coal tar and olive oil, coal tar and liquid soap, and non-diluted coal tar at 1, 5, 10, and 20 days after transplantation of the embryos in each of the embryomata produced by injecting chopped up 12, 13, 14, 15, 16, and 17-day-old rat embryos. The results of the effect of coal tar on embryomata produced by injecting chopped-up embryos of different ages is tabulated in Chart 1.

After several embryomata each being produced by injecting 2 chopped-up 15-day-old rat embryos, were 30 days old, I injected one cc. of undiluted coal tar every ten days into each of the tumors. An animal was killed and autopsied every thirty days

following the first injection of the tar and a study of the growth changes in the tumor as well as a general study of the organs was made. The findings were as follows:

One tumor was removed two days after the first injection of the coal tar. In the connective tissue areas the fibrils are separated and flattened about the droplets of the tar. In many

CHART 1



The size of tumors produced by injecting coal tar into experimental embryomat produced by injecting chopped up embryos of various ages. In one half of the series injections of tar were begun ten days and in the other half twenty days after the transplantation of embryonal tissue.

places there is definite increase in fibroblasts in the fibers about the coal tar. Whenever the tar has come in contact with the epithelium of one of the epithelial lined cysts, the epithelial cells have migrated in large numbers to surround the droplets and to become dispersed between distant droplets of coal tar. Near the epithelial layer these cells are collected in dense masses. Many mitoses are already visible in these dense masses (Fig. 6). (Fig. 11 is a drawing of this same section).

The next tumors studied were removed 30 days after the first injection of coal tar. These tumors were growing. How much the coal tar affected the growth of the tumor was not determined.

Much of the normal growth of these embryomata is due to a filling of the cysts with more fluid. Microscopically, the reactions about the tar is of interest. In this tumor the tar, as in the former case, is found both in the connective tissue portions and near the epithelial cells lining many of the cysts of the embryoma. These cysts in the embryomata untreated by tar are lined chiefly by squamous epithelium of the skin or by cylindrical epithelium as in the intestine. Where the coal tar lay near a layer of squamous epithelium the picture was similar to that in the first experiment except much more advanced. The epithelial layer has thickened to become a tumor composed of long papillae which contains many pearls. The papillae extend downward into the wall of the cyst and encapsulate the coal tar like individual cells had done in the previous experiment (Fig. 7). Histologically, a true prickle cell carcinoma had apparently developed. At the earlier period only the basal cells had moved down. They had remained more separated. The picture was more like that of an atypical basal cell tumor (Fig. 11.)

In the purely mesenchymatous areas the mesenchyme cells have increased greatly in number in the connective-tissue fibers which have encircled the drops of coal tar. The number of connective-tissue fibers, on the other hand, about these drops of tar has not increased. They are the original fibers of the tissue pushed together by the injection of the mass of tar (Figs. 4 and 5).

In the tumor removed from the rat killed 60 days after the initial injection, the coal tar was found only in the areas of connective tissue. It is in the form of one large mass. Each new injection had been made in the area of the previous injection. The capsule about the mass of coal tar is composed entirely of mesenchyme. It differs from those of the former cases only in that the cells around the fibers are greatly increased in number. They show shriveling and early degenerative changes. No mitoses are seen.

In the tumor removed at 90 days the coal tar had been placed entirely in the mesenchyme or connective tissue parts of the

embryos. At this time the connective-tissue fibers surrounding the droplets of tar are not denser than in the tumor removed 2 days after the first injection. The fibroblasts on the other hand, have greatly increased in number in these capsules until they form almost continuous masses in many regions. Mitoses are seen in these cells in many parts of the section. The cells are not shriveled nor have they degenerated. They are large, well formed and contain nuclei rich in chromatin, as in Fig. 5.

No more tumors were removed until 210 days following the initial injection of the coal tar. The later injections in these older tumors did not reach the site of the earlier injections. In the section of the tumor removed at 210 days the masses of tar show a reaction about them like those seen in adult connective tissue 180 days following the initial injection. In places throughout this tumor the capsule surrounding the droplets is composed almost entirely of fibroblasts. In other places the capsule is hyaline containing only a few shriveled fibroblasts.

A section from a tumor removed 240 days after the first injection contained a large mass of coal tar embedded in a narrow fibrous capsule. This capsule is composed largely of a dense mass of fibroblasts. Mitoses are seen in several places where these cells are more numerous.

In other cases the tar which had remained for a very long time in the tissue was studied. After a single injection of tar had remained in the tumor for 260 days the capsules had undergone hyaline changes. The increased number of fibroblasts as noted previously had disappeared in this process of hyalinization. The tar in this section is not as black as at an earlier time, Fig. 10.

In practically none of these experiments with coal tar have I seen any increase in the leucocytes or wandering cells about these drops of coal tar. The tar acts entirely on the fixed tissue cells. When introduced into the tissue beneath the epidermis there is a rapid piling up of cells about the droplets. The epithelial cells migrate at first to surround and encapsulate the drops of tar. Even in the earlier stages one sees a dense mass of these cells about the droplets of tar. In the earlier stages the

cells nearest the tar show a shrinkage in size and often a degeneration. Later, proliferation becomes more active until a considerable tumor of a prickle cell type, is often developed.

In very few cases have I succeeded in introducing the second and following portions of coal tar into the same locality as the first. Most of my experiments represent only the effect of one or a small number of doses of coal tar, separated but close together.

In the connective tissue the tar produces the same changes as in the epithelial structures. When introduced into the tissue the fibers of connective tissue are pushed apart to form a capsule about the drop. These do not increase in number. In one or two cases where the coal tar produced an early cellular necrosis, leucocytes and exudate accumulated. A thicker and more dense fibrous capsule may have developed in such cases. In most cases the capsule is merely the original fibrous tissue pushed apart by the introduction of the tar and the later shrinkage of the tissue.

The action of coal tar is to draw fixed tissue cells to it and to destroy previously existing intercellular fibrils rather than build them. The endothelial cells of the blood vessels are attracted apparently as readily as the fibroblasts and epithelial tissue. About droplets of tar one frequently sees the whole tissue shrink in size and pucker. This is shown in Fig. 9.

In none of the sections have I noticed any evidence that coal tar stimulates the cells to grow. Coal tar acts to draw cells to it away from their blood supply. It acts on the blood vessels to destroy them through the attraction of their endothelial cells from them. It acts to cause a degeneration of the intercellular fibrils. Near the more cellular epidermis large numbers of cells are so attracted to the tar. Proliferation is seen in these crowded masses of cells. In the less cellular connective tissue of adults fewer cells are collected together. In the more cellular mesenchymatous tissue of embryos more cells are collected about the droplets. Only in these more cellular masses is there any evidence of cellular proliferation. The number of cells collected in each case is proportional to the original number of cells in the

tissue about the tar and the amount of new tar introduced from time to time. The activity of the tar always decreases. A single drop soon becomes inactive. The tissue formed about it, unless it is very cellular, suffers slow degenerative change of a hyaline nature.

Cachexia and the Migration of Coal Tar in the Tissues

Burrows (25) in a series of experiments on body fats has been able to give direct proof that the taking in of fat by body cells is the result of the adsorption of *ergusia* by the fats. He has found that fats in the blood stream inhibit cell migration as they are dragged into the cell. Droplets of fat taken from the cell may cause coagulation, stick to fibrin and repelled cells while they are losing the *ergusia* which they have adsorbed. These fat droplets from the cells migrate in a manner similar to cells in the culture.

In the body a substance which takes up *ergusia* should also cause marked nutritional disturbances of the animal. In a special series of experiments it became of interest, therefore, to study the later behavior of coal tar in the tissue and its general quantitative effect on the animal. Earlier authors had noted that cancer is not induced by single injections of tar. It was interesting to me to note that single injections of tar into the subcutaneous tissue of rats cause only hyaline scars while more cellular masses result in the areas where more and more tar was successively introduced. The action of the single injections of the tar was always limited like any substance becoming saturated and thus becoming inactive. In a certain number of these tumors the tar disappeared. In others it remained within its capsule.

In the experiments cited above I injected only 1 cc. of tar at a time. Larger doses of coal tar (10 cc. in an adult rat) I find kills the animals within 2 to 3 days. Slightly smaller doses cause an intense cachexia with eventual death.

As I pointed out above, Burrows has found that the proteins and fats of the blood vessels do not contain *ergusia*. They adsorb it readily from the cells. In sections of coal tar tumors removed after thirty days the tar was found often in the blood

vessels. In some cases this tar had been introduced directly. In others it had migrated apparently into these vessels at a later time. Where it was directly introduced the endothelial cells were degenerated and there was an increase in cells about the vessels. In older specimens where it had migrated into these vessels it had little effect on the endothelial cells.

In other experiments I removed the hair from the axillary region of a series of white rats, scarified the denuded area and painted it with coal tar to determine if the product would be transported by the capillary or lymphatic route. After 30 days (5 paintings) the area kept denuded by application of sodium sulphide and scarification repeated each time, lymphatic like capillaries were found filled with tar.

In a large number of rats I have studied carefully the behavior of the tar grossly, in specimens where it was injected subcutaneously. In very few of the tumors removed early did I observe any migration of the tar. It was after several weeks that I found it invading the veins (Fig. 12). In a few cases it migrated in these vessels to the lungs and distant organs.

Thus, in another series of animals I injected 2 cc. of olive oil and india ink, subcutaneously in the axillary region to further determine the transportation of these products through vascular routes. The resultant phenomena was the same as noted in the previous experiments. In the subcutaneous areas the tar invariably entered the veins. On the surface of the skin it sometimes entered the lymphatics, as the cells of carcinoma tend to migrate into the lymphatics rather than in the veins.

Doctor Tobias working in conjunction with me in this laboratory a year ago studied the action of intramuscular injections of large amounts of coal tar. In a small series of adult rabbits (2,600 to 2,800 gms. in weight) he injected 15 to 40 cc. of coal tar subcutaneously into the costal and dorsal regions of these animals. Death resulted within 2 to 4 days after the injection. Autopsies showed an extensive infiltration at the site of injection and throughout the subcutaneous tissue. In one case acute inflammation and edema was noted. No puncture wounds in the abdominal wall were observed in any of these cases, yet the

peritoneum and visceral cavities often became filled with the coal tar (unpublished notes).

DISCUSSION AND CONCLUSION

Previous authors have shown definitely that sarcomata and carcinomata may be produced by injecting coal tar. This coal tar is the cause of the cancer only in so far as it induces changes which allow the cells to grow. Cancers once induced by coal tar continue to grow to the destruction of the animals independent of the tar.

In previous work Burrows had failed to find the cancer cell to be different from normal cells of the same type. In the tissue culture, body cells will not grow when merely supplied with ample food and oxygen. Growth depends also on their being crowded together so that the *archusia*, a certain product of their metabolism, can accumulate about them. An active circulation or a separation of the cells either prevents the formation of the *archusia* or its concentration about the cells. Crowded masses of cells he found not only able to grow, but they prey readily on other cells for the substances necessary for their growth. Among the numerous experiments carried on in this direction he studied the effect of continuous pressure on areas of the normal skin of rats. This pressure always led to atrophy, degeneration and ulceration. In other experiments he first stimulated the epithelial cells of the epidermis and glands of the skin to grow and increase per unit area in the tissue. Continuous pressure on such a skin was associated with a delay in the atrophy and ulceration or it led to the production of cancerous ulcers. Cancer as he saw it, was the result of anything which led to the proper increase in cells in a given area and a proper reduction in the blood supply of this area. The energy of body cells is at all times proportional to the concentration of their *archusia* about them. This concentration is maintained or controlled entirely by the environment.

The problem to solve in these experiments is how does coal tar act to produce cancer. In the above experiments it has been shown that the primary changes are a movement of fixed tissue

cells towards the drops of viscid tar. The tar does not act to stimulate growth but to attract cells to it and away from their blood supply. Growth is a secondary phenomenon which intercedes only when the cells become sufficiently crowded about the coal tar and their vascular supply sufficiently reduced by a pulling away of the endothelial cells of adjacent capillaries.

The coal tar acts to attract cells only in that it dissolves the *ergusia* of the cells. Its action continues until it becomes saturated with this substance. It may then be inactive in the tissue or if conditions are suitable it may migrate into veins which contain little of the *ergusia* as any substance containing a surface tension lowering substance may migrate from points in the medium containing a high concentration of the surface tension lowering substance to points of lower concentration of this substance.

In the sparsely cellular connective tissue of adults it is very difficult to collect together sufficient cells to produce growth. In the more cellular mesenchyme of embryos more densely cellular masses are easily brought together as they are easily produced in the more cellular epithelial structure of both adults and embryos. This failure to pull cells from the sparcely cellular tissue of adults is not due entirely to the few cells present but to the fact that they become exhausted of their *ergusia* and degenerate. In a few animals densely cellular masses were accumulated by drops of tar. Tar becomes toxic for the whole animal and leads to cachexia through its ability to thus draw the *ergusia* to it.

It is not surprising therefore that the authors should have found that cancer can be produced only by continuous application of coal tar over a long time. It is again not surprising that carcinomata are more easily produced in adults than sarcomata. The hyaline scar tissue noted by Mook and Wander about drops of paraffin oil in patients is the normal outcome of single or even repeated injections of coal tar into the subcutaneous tissue of adults.

The action of coal tar is purely physical in its nature. Kennaway (27) has recently attempted to find a growth stimulus in

coal tar. He failed in these endeavors but finds that coal tar distillates coming off at or above 255° C are the only ones which produce cancer in animals or in man. As I have pointed out above, it is necessary for cells to be so drawn together that the adsorbing medium be held in one place with sufficient tenacity to overcome the inertia of the moving cell. More mobile oils are drawn to the cells. Recently Burrows and Johnson (unpublished notes from this laboratory) have found that vegetable oil, such as corn oil, will produce the same reaction in tissue as coal tar. There is no reason to believe that the corn oil contains chemically active substances similar to the coal tar while it may have the same solvent action.

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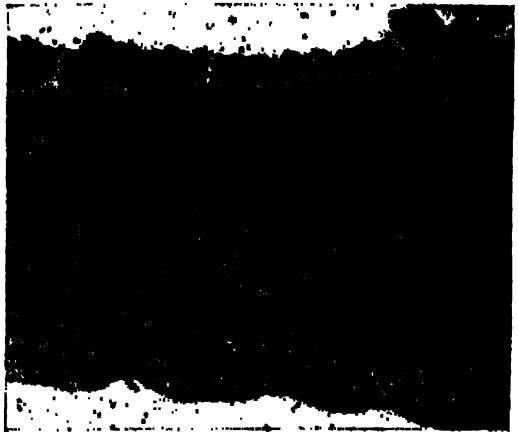
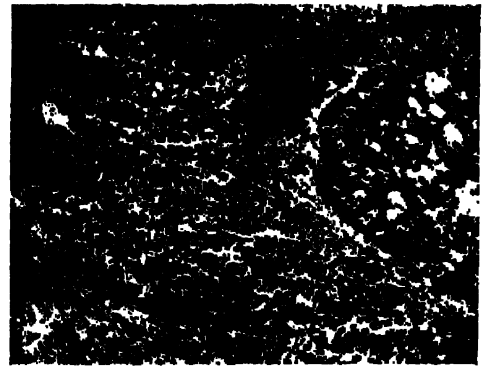
PLATE I





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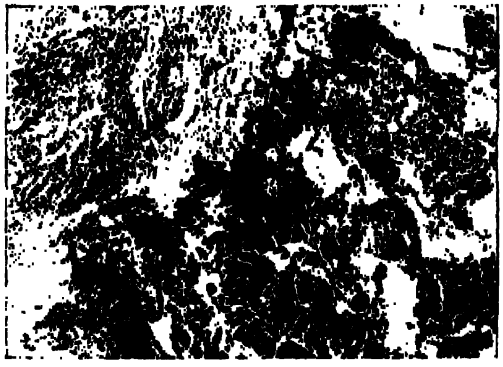

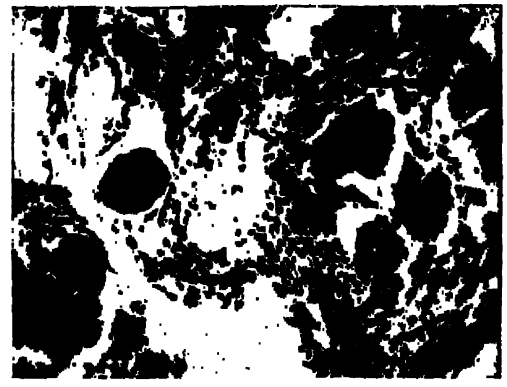

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PLATE II

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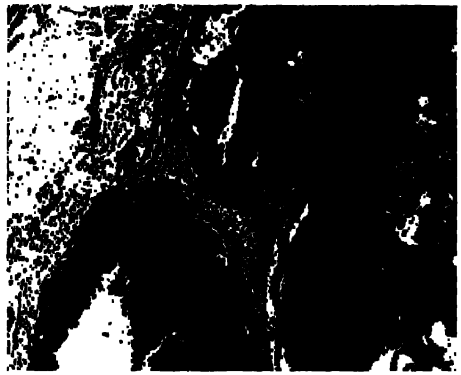

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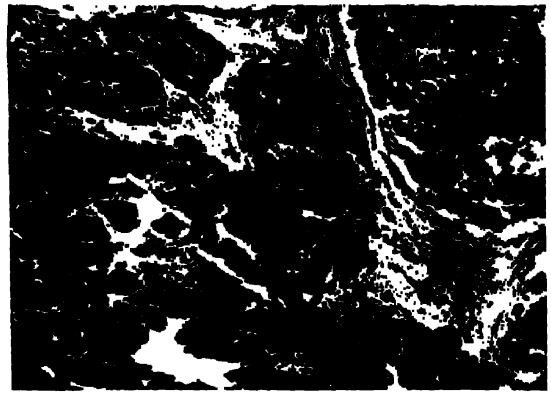

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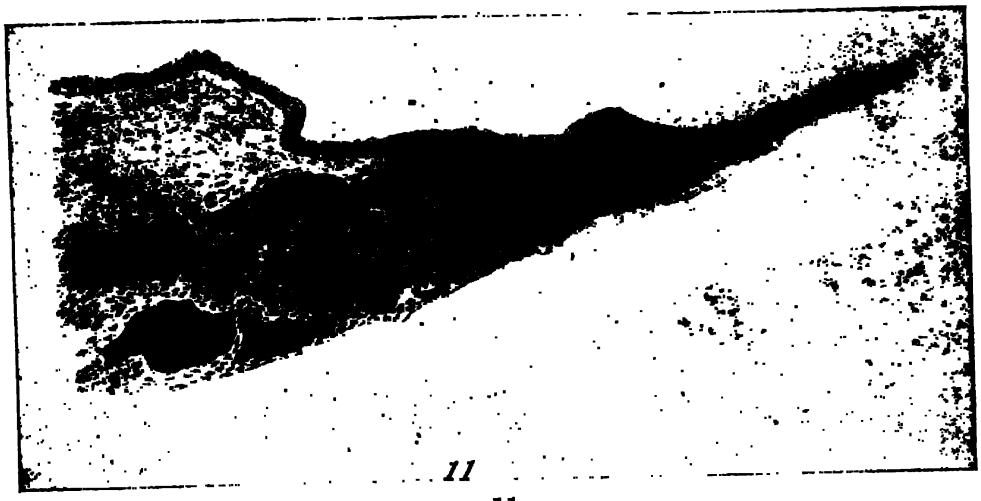
PLATE III



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EXPLANATION OF PLATES

PLATE I

FIG. I. A low power microphotograph of a section of adult rat tissue 4 days after an initial injection of coal tar.

FIG. II. A low power microphotograph of a section of adult rat tissue 18 days after an initial injection of coal tar.

FIG. III. A high power microphotograph of a section of adult rat tissue 90 days after an initial injection of coal tar.

FIGS. IV and V. A low and high power microphotograph of a section of an experimental embryoma 30 days after injections of coal tar.

PLATE II

FIG. VI. A low power microphotograph of a section of an experimental embryoma 2 days after injections of coal tar.

FIG. VII. A low power microphotograph of a section of an experimental embryoma 30 days after injections of coal tar.

FIG. VIII. A low power microphotograph of a section of adult rat tissue 30 days after a single injection of coal tar.

FIG. IX. A low power microphotograph of a section of adult rat tissue 180 days after injection of a few drops of coal tar.

FIG. X. A high power microphotograph of a section of adult rat tissue 260 days following a single injection of coal tar.

PLATE III

FIG. XI. Drawing of section shown in Fig. VI.

FIG. XII. Drawing of gross specimen—60 days after injection of coal tar under skin of adult rat.

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THE RELATION OF THE VITAMINS TO THE REACTION INDUCED BY COAL TAR IN THE TISSUES OF ANIMALS.

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PLATES 7 TO 9.

(Received for publication, May 26, 1925.)

As the important problem in the study of cancer is the nature of conditions which regulate the normal growth of cells in the organism, it becomes necessary to study, in relation to tissue changes brought about by coal tar, the conditions and substances which promote normal growth. In previous papers (1) I have studied the action of coal tar in adult and embryonic tissues of the rat. These animals were fed on a balanced dietary throughout the experiments. Following the same procedure and technique, in so far as the introduction of coal tar into the tissue is concerned, I have modified the dietary in the feeding of a series of rats and studied the tissue changes produced by coal tar as affected by these conditions.

In the previous paper it had been shown that the chief effect of coal tar on the cells and organism is destructive rather than constructive. When drops of coal tar are injected into the subcutaneous tissue they attract the tissue cells to them from a wide area about them. As these cells leave the blood vessels and intercellular fibrils of the connective tissue the intercellular substances suffer not only a loss of their cells, but hyaline-like changes. The cells as they migrate to the edge of the tar round off, their nuclei and cytoplasm stain less sharply. Many of the first cells to arrive suffer a complete granular degeneration.

When a sufficient quantity of coal tar is injected into the subcutaneous tissue of an animal, the cells not only undergo this migration and show these degenerative changes, but the whole animal suffers a loss of weight, cachexia-like changes, and often an eventual death. The changes occurring in these animals as they are seen in the gross, are not unlike those that occur in animals with rapidly growing malignant tumors. Many of the earlier authors had already noted that lipoid solvents act to cause a death of the cells rather than any stimulation of them.

Such observations are seen in the articles by Bullock and Rohdenburg (2) and Champy (3). These authors had thought, therefore, that the action of these substances in the production of cancer is largely one of destruction with secondary regeneration. How the substances cause the degeneration and effect the general nutrition of the animal had not been determined, however.

The action of a single drop of coal tar is always limited. Its acts to produce the changes noted above for only a short period of time. Its action is greatest at first, then it gradually wanes. It acts, therefore, in the tissue like a substance which dissolves some important constituent of the cell. Those cells which are drawn to the edge of these droplets of tar surround it as a continuous cellular layer one or many cells thick. After the tar has ceased to be active those of the cells which are not completely destroyed become active again. Their nuclei and cytoplasm stain more sharply. In the sparsely cellular tissue of adults a single injection of coal tar rarely ever draws more than a few cells to its periphery. In the more densely cellular mesenchyme of embryonic tissue and near the epidermis of adults many more cells are attracted. Where a large number of cells are crowded together about the tar they show growth and multiply as they recover from the primary effects of tar. Where only a few are attracted to the edge of the drop, they never show any growth, but slowly develop new intercellular substances and the whole eventually reverts to a hyaline scar.

The cells that more to the edge of the coal tar are the cells which are present in the tissues immediately about it. They are endothelial cells, fibroblasts, epithelial cells, leucocytes, and other wandering cells. With each new addition of coal tar more and more cells are attracted to the tar. With these repeated applications of tar the tissue about suffers much more exaggerated changes. Most or all of the cells in the nearby tissues are drawn to the tar. The surrounding tissues assume a hyaline appearance peculiar to tissue about actively growing cancers. In the more densely cellular masses accumulated by frequent applications of tar, growth and mitosis of cells intervene and cancer develops.

In studying these various effects of coal tar, it has been interesting to note that different animals respond differently to the injection of this substance. In a number of cases all the cells degenerated when they moved to the tar. In other cases they withstood the action of the tar and formed a dense cellular collar about each drop of this substance. Other authors have noted similar variations in these cellular responses in that cancer developed more rapidly in some of their animals, but no one has explained these variations. It became of interest, therefore, to investigate them in regard to the general state of nutrition of the animals and the diet they received.

For all these experiments young healthy actively growing animals have been used. The diets given them were modifications of those used by former workers on vitamins—Goldblatt (4), Gross (5), Zilva (6), Hume and Smith (7), Bond (8), Eddy (9), and McCollum (10). It was important to note carefully if disturbances

in nutrition and the arrest or retardation of growth was due to vitamin deficiencies in these diets, or due to non-palatability, or decreased consumption of food. Results due merely to lack of food intake were discarded.

The chief immediate index to the effect of any particular diet is the gain or loss in weight of the animal. Repeated weighings were carried out during the course of the experiment. The injections of coal tar into the animal were regulated as to time and amount on the basis of the weight curves and the study of tissue changes, after determining the effect of similar quantities of tar in a series of animals fed on an ordinary normal diet.

Diets.

1. Balanced ration.
   - Egg albumin scales (finely ground) ..... 25 gm.
   - Potato starch ..... 40 "
   - Butter ..... 15 "
   - Salt mixture (McCollum) ..... 5 "
   - Lemon juice ..... 5 cc.
   - Vegex* ..... 5 gm.
2. Moderately high in vitamin A.
   - Egg albumin scales ..... 25 gm.
   - Potato starch ..... 40 "
   - Butter ..... 25 "
   - Salt mixture ..... 5 "
   - Lemon juice ..... 5 cc.
   - Vegex ..... 5 gm.

2, *a*. High in vitamin A.
   Cod liver oil, 5 cc., added to (2).

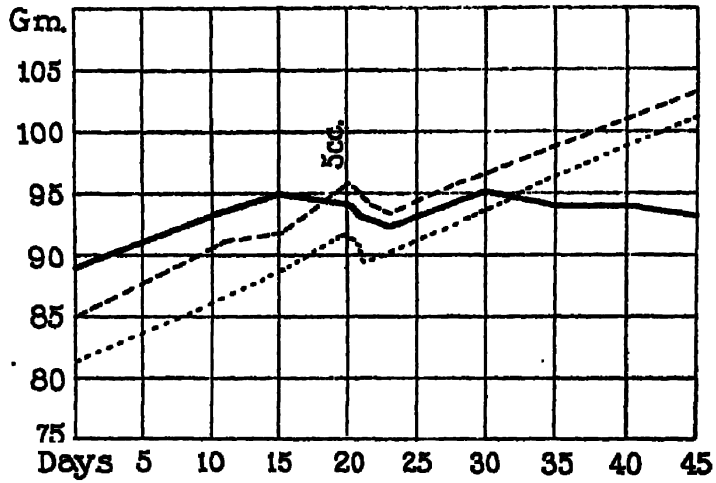
3. Deficient in vitamin A.
   Crisco substituted for butter in (2).
4. High in vitamin B.
   - Egg albumin scales ..... 25 gm.
   - Potato starch ..... 40 "
   - Butter ..... 15 "
   - Salt mixture ..... 5 "
   - Lemon juice ..... 5 cc.
   - Vegex ..... 10 gm.
5. Deficient in vitamin B.
   Vegex not included in the ration (4).
6. High in vitamin C.
   - Egg albumin scales ..... 25 gm.
   - Potato starch ..... 40 "
   - Butter ..... 15 "
   - Salt mixture ..... 5 "
   - Vegex ..... 5 "
   - Lemon juice ..... 10 cc.
7. Deficient in vitamin C.
   Lemon juice not added to the ration (6).

* Vegex is the source of vitamin B and a product furnished by the vitamin Food Company, Inc., Westfield, Mass.

EXPERIMENTAL DATA.

150 white rats were used in this experiment. They were divided into series, each series being made up of animals of practically the same age and weight. All were from the regular brother and sister strains of stock that has been bred in our laboratory during the past 5 years.

For the study of tissue changes around the particles of coal tar introduced under the skin, animals from each series were killed and autopsied at regular intervals of 2, 15, 30, 45, and 60 days after the injections of tar. A number of animals were kept for a longer period of time and some were autopsied and sections made of the tumor when the animal died as result of the deficiency diet. In other cases biop-



--High vitamin B diet.
—Low vitamin B diet.
·-Balanced diet.

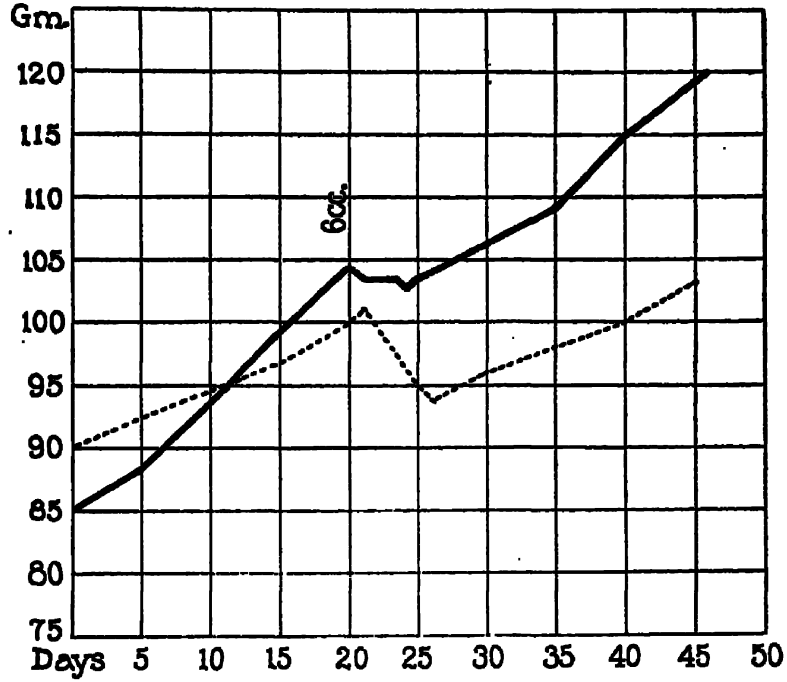
TEXT-FIG. 1. Curve showing changes in weight before and after the injection of 5 cc. of coal tar into three rats on a high vitamin B, deficient vitamin B, and balanced diet respectively.

sies were made from animals under ether anesthesia. A certain number in each series will be kept for a long period of time and findings reported later.

Discussion of Weight Curves.

The weight curves are practically self-explanatory and need emphasis only in regard to a few points of particular interest. Taking into

consideration the weight of the animal and the amount of coal tar injected over a period of time, the weight curves of the animals on a deficient vitamin B and a high vitamin B diet are practically identical (Text-fig. 1). There is a definite decrease in weight after each injection of coal tar and the composite growth curve follows a line almost parallel to the base line. The animals on a balanced dietary lost



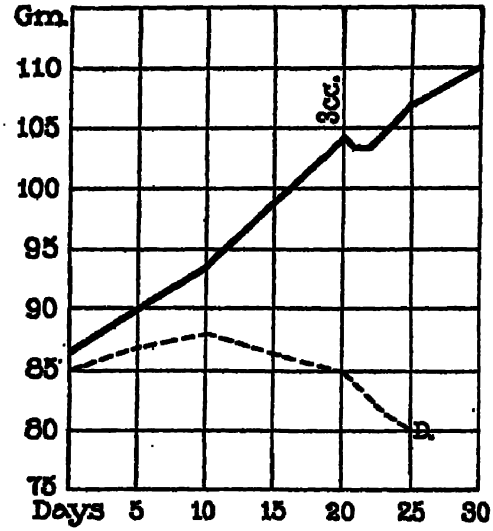
TEXT-FIG. 2. Curve showing changes in weight before and after the injection of 6 cc. of coal tar into two rats on a high vitamin A and a stock (balanced) diet respectively.

weight after each injection of coal tar, but they increased in weight gradually, not as rapidly, however, as an animal that has not been injected with coal tar (Text-fig. 2).

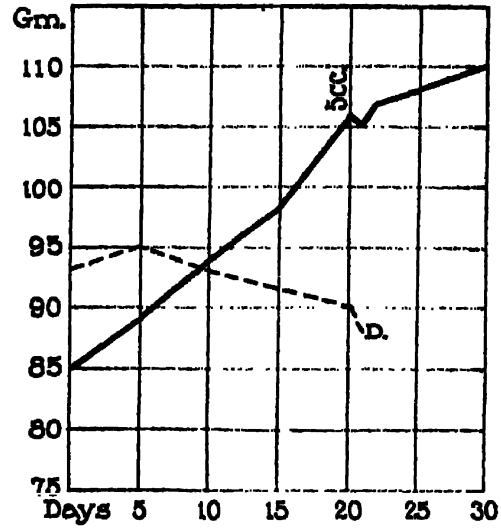
Animals fed the deficient vitamin A diet lost weight rapidly after the injection of coal tar, even after as small amount as 1 cc., and when the amount was increased to 3 cc., the animal died within a few days or even earlier. On the other hand, in the series receiving a high

vitamin A ration the weight curve was little affected by the injections (Text-figs. 3 and 4). 10 cc. of coal tar did not always kill these animals.

A deficiency in vitamin C gives a weight curve similar to that of a balanced dietary. The withdrawal of this vitamin has no effect on the reaction of the animal to the tar.



TEXT-FIG. 3.



TEXT-FIG. 4.

——————High vitamin A diet.
– – – – – – –Low vitamin A diet.

TEXT-FIG. 3. Curve showing changes in weight before and after the injection of 3 cc. of coal tar into two rats on a high vitamin A and a low vitamin A diet respectively. Rats on a low vitamin A diet died (D.) quickly after injection.

TEXT-FIG. 4. Curve showing changes in weight before and after the injection of 5 cc. of coal tar into two rats on a high vitamin A and low vitamin A diet respectively.

Discussion of Tissue Changes.

In a previous paper (1) I discussed the tissue changes brought about by the introduction of coal tar into adult and embryonic tissue. The series of animals on the balanced ration dietary in this experiment presented tissue changes of the same character. There may be an earlier cellular degeneration and hyalinization in the tumors taken from the animals fed on the diet deficient in vitamin B but it is not marked. There is no cell activity after the 10 day period (Fig. 1).

In the animals fed on a high vitamin B diet, the collar of fixed tissue about the coal tar is more dense. Cells have been attracted from a

wider zone than in the case of animals fed on a balanced ration or one deficient in vitamin B. Activity is not marked, but the cellular content of the collar is greater than can be accounted for by a mere drawing in of cells from the surrounding tissue. From 15 to 20 days after the injection of tar, degeneration and hyalinization have taken place (Fig. 2).

The striking feature of the tissue changes brought about in the deficient vitamin A animals is the sparcity of cells in the zone about the coal tar. The cells have been attracted from the surrounding tissue, but these cells have suffered a marked degeneration in their migration to the tar (Fig. 3). In only one case did they show even slight activity. All of them shrivel and break up at an early period.

In the animals fed on the normal diet of the laboratory, on the specially prepared balanced diet, and one deficient in vitamin A or B, or high in vitamin B, the coal tar tends to remain in large drops as it was injected into the tissue. It is quite different in this regard from the corn oil described by Burrows and Johnston (11). In most cases the corn oil breaks up quickly into numerous small droplets.

In the animals fed on a diet high in vitamin A this picture changes. The coal tar breaks up quickly into a large number of small droplets (Figs. 4 and 6). These are found scattered in a densely cellular tissue. In a few of the sections removed from animals fed a balanced ration the periphery of the drop of coal tar is partially broken up by the cells. The picture is quite different, however, from the complete breaking up of the drop in the animals fed a diet high in vitamin A. The cells about these droplets are large and contain well formed, sharply staining nuclei. Many of them are dividing by mitosis. This picture is most marked in the 15 to 30 day specimen (Fig. 5). After this period certain portions of the tissue surrounding the droplets undergo hyalinization, cellular activity continuing only in scattered cell nests. These cell nests present the picture of early sarcomatous changes. The collars of cells about the individual droplets are much larger than in animals fed on other diets. They are often thirty cells thick.

DISCUSSION.

In my previous paper (1) I had shown that coal tar acts on the organism to cause cachexia-like changes. Drops of coal tar placed in the

tissue attract the fixed tissue cells to them from the surrounding tissues. They do not stimulate these cells to grow but the cells suffer partial or complete degeneration in this reaction. This action of the tar is not continuous, but limited to a short period of time. After the tar has ceased to be active those cells which have not degenerated completely recover from the action of the tar. Where the cells have become densely packed together about the tar active growth and division of these cells intervene and cancer develops. Where only a few cells are collected by the tar or only a few survive its action they form only a hyaline scar. There was no evidence that the tar acted, at any time, to stimulate the cells to grow. It acts as a simple solvent of lipoid elements in the cell. The degeneration in the cells and the cachexia may be the direct result of the removal of these lipoids by the tar.

Burrows (12) in his studies of body cells in the tissue culture has shown that these cells have many features in common with yeast and other unicellular organisms. They can grow independently as in cancer only when they are crowded together in a stagnant area where certain primary products of their metabolism can accumulate about them. They differ from many of the other unicellular organisms in that they have no special mechanism for migration. They migrate by liberating a surface tension-lowering substance absorbed only by proteins and fats. They can move only into proteins and towards larger droplets of fat which absorb this surface tension-lowering substance. The primary substance which must concentrate about these cells for them to become active is a water-soluble substance formed by them in proportion to the oxygen absorbed. It has been called the *archusia* (S). The surface tension-lowering substance has been called the *ergusia*. This latter substance is not liberated by the cell under all conditions, but only when the archusia (S) reaches a certain concentration (S_2). It is not re-formed in the cell until the archusia reaches a still higher concentration (S_3) or when the cells grow and divide. Coal tar dissolves the ergusia.

Body cells cannot retain the archusia. They can grow only when it is extracted from other sources and added to the medium or when they are crowded together in a stagnant medium from which it cannot escape. In the normal organism the archusia does not reach a con-

centration consistent for growth and re-formation of the ergusia. The question arose as to how these cells in the normal organism survive under such conditions. Is their mechanism for growth different from that in the culture, or do they obtain a supply of these substances from other sources? It seemed possible that certain of the vitamins were none other than these two essentials supplied by other growing animals and plants. To test this conception it became of interest to see the effect of coal tar on animals fed on a diet rich in one or the other of the various vitamins. These studies have shown that a diet rich in the water-soluble vitamin B stimulates the growth of these cells just as the archusia stimulates the cells in the culture, while the fat-soluble vitamin A protects the animal and the cells against the destructive action of coal tar or the loss of the ergusia to it.

In the light of these facts it has been possible, therefore, to draw the following conclusions.

SUMMARY AND CONCLUSIONS.

Drops of coal tar introduced into the subcutaneous tissue attract the fibroblasts, endothelial and other cells to them. These cells suffer degenerative changes through this action of the tar and the animal suffers cachectic-like changes and death from large doses of it introduced into the subcutaneous tissue. This action of the coal tar is limited to a short period of time, after which it becomes inert. The cells which have been drawn to it and which have not completely degenerated then slowly recover. Where large numbers of these cells are drawn to the tar they grow and divide after recovering from the initial effects of the tar. Cancer may develop. Where only a few cells are drawn to the tar they lay down intercellular fibrils and a scar eventually develops. Vitamin A fed in more than ample quantities to these animals protects the animals and the cells against the toxic action of the tar and stimulates and prolongs their secondary growth. Vitamin B stimulates the secondary growth of these cells. This action is limited in extent and time. It is followed by an early degeneration and hyalinization of the tissue.

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11. Burrows, M. T., and Johnston, C. G., *Arch. Int. Med.*, 1925 (in press).
12. Burrows, M. T., *Proc. Soc. Exp. Biol. and Med.*, 1924–25, xxii, 241.

EXPLANATION OF PLATES.

The figures are photomicrographs of sections of tissue of adult rats, showing the cellular changes about drops of coal tar. Each series of animals was placed on the different diets 30 days before the injection of the tar or oil.

PLATE 7.

FIG. 1. Moderately low power photomicrograph showing tissue changes 30 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet deficient in vitamin B.

FIG. 2. Moderately low power photomicrograph showing tissue changes 30 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet high in vitamin B.

PLATE 8.

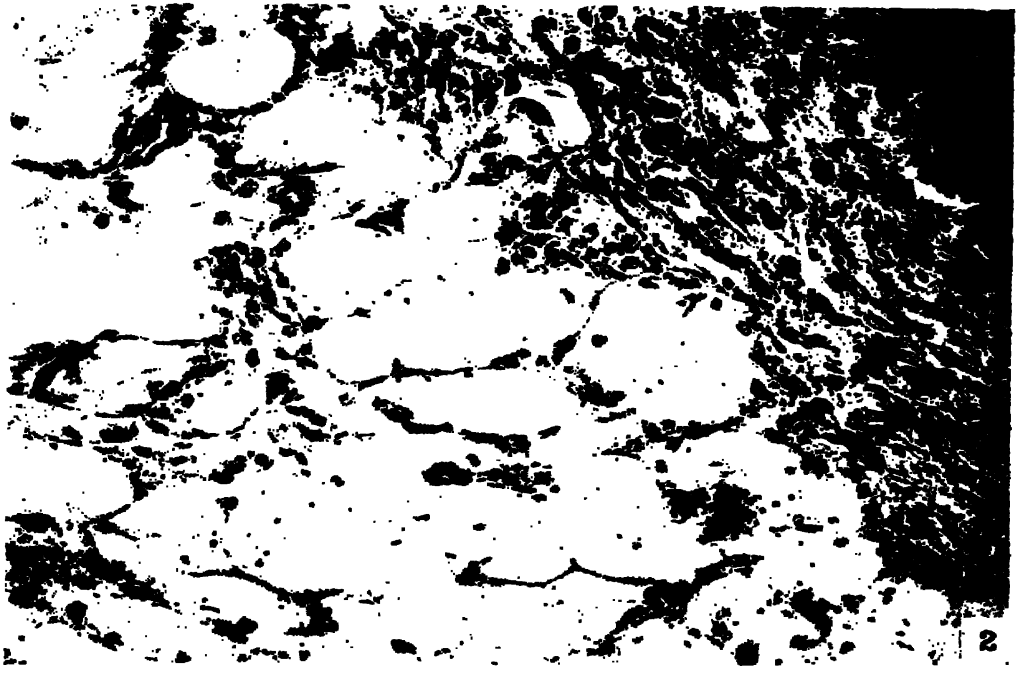
FIG. 3. Moderately low power photomicrograph showing tissue changes 28 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet deficient in vitamin A.

FIG. 4. Moderately high power photomicrograph showing tissue changes 30 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet high in vitamin A.

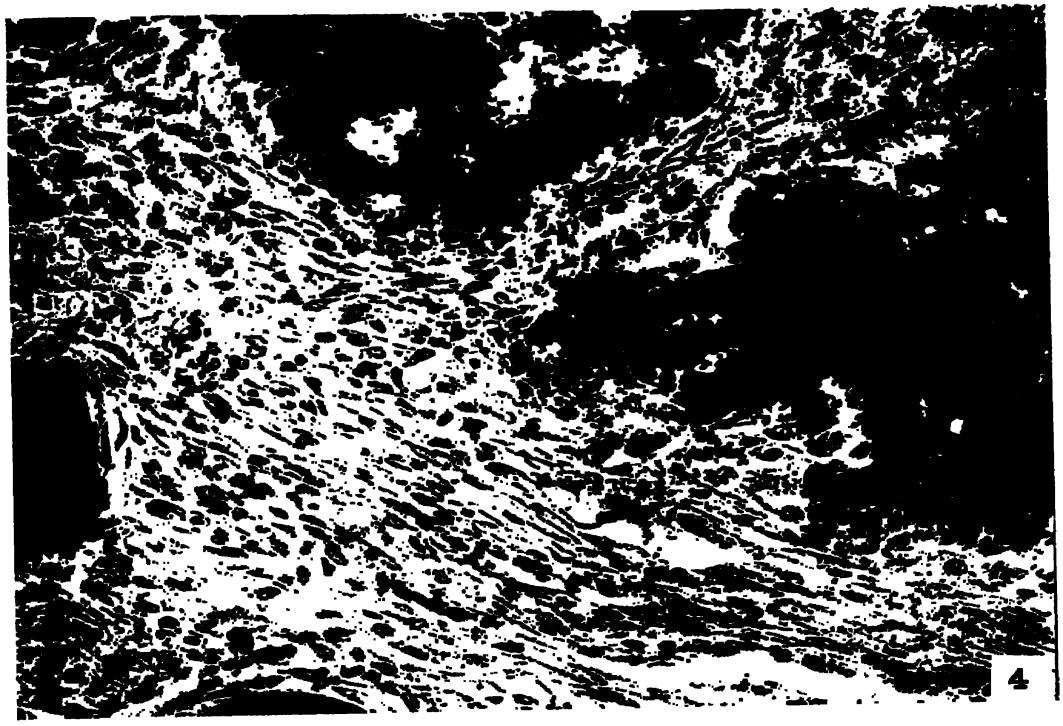
PLATE 9.

FIG. 5. A higher power photomicrograph of the same section as that shown in Fig. 4. The striking picture is the numerous mitotic figures as compared to sections taken from the rats on the other diets.

FIG. 6. A low power photomicrograph showing the breaking up of the coal tar into small droplets in the tissues of a rat on a high vitamin A diet. The section was taken from the rat 30 days after injection of the tar.



(Jorstad: Reaction induced by coal tar in tissues.)



(Jorstad: Reaction induced by coal tar in tissues.)

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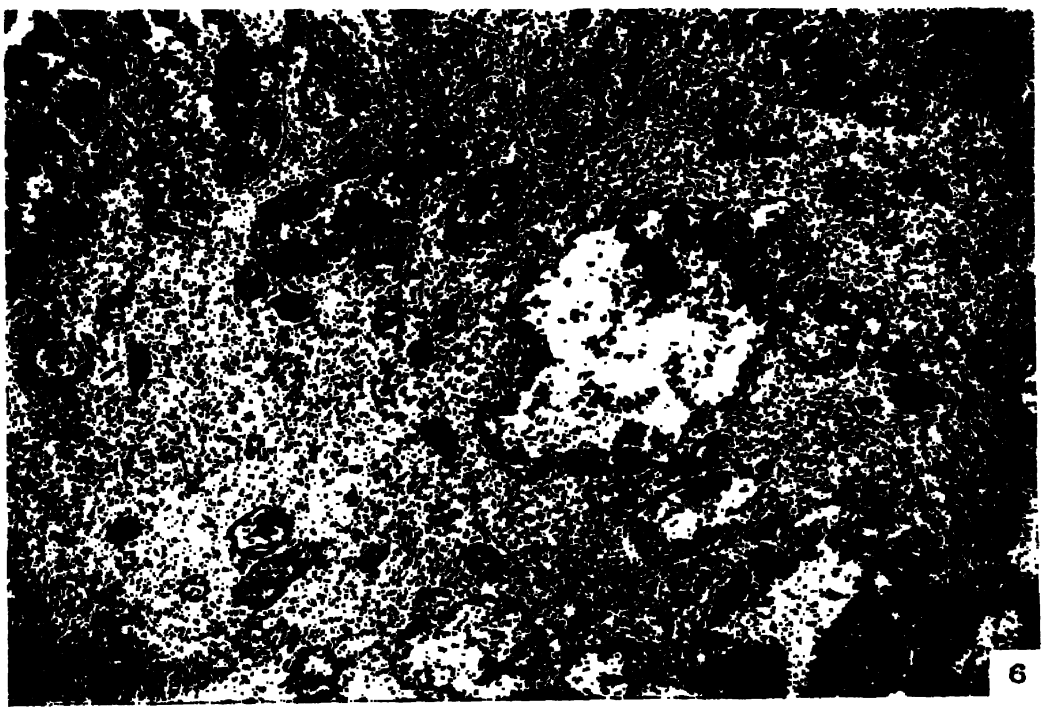

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(Jorstad: Reaction induced by coal tar in tissues.)

becoming saturated with the *ergusia*. It is on this account that single drops of tar cannot produce cancer. Several applications are necessary to draw sufficient cells together for them to retain sufficient growth energy to overcome the resistance of the body.

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August, 1925, pages 338-339

Since these cells cannot retain their *archusia* except in stagnant environments an independent growth of them depends on a crowding of the cells and stagnation. Such are the conditions existing only in cancer. Coal tar dissolves the *ergusia* of the cells. Viscid drops of this substance placed in the tissue draws the tissue cells to it away from their intercellular substances and blood vessels. In doing so the cell suffers certain degenerative changes from the loss of their *ergusia*. Jorstad has shown that coal tar produces cancer only in that it thus builds about itself a dense mass of cells poor in blood vessels. It does not stimulate the cells to grow, but as they become massed together and the *archusia* which they form stagnates about them, they assume the power of independent growth.

In the normal organism the cells cannot grow unless supplied with *archusia* from other sources. We have noted that the cell's activity is dependent on the presence of two substances, the *archusia*, a water soluble substance, and the *ergusia*, a fat soluble substance. These correspond, as far as these solubilities are concerned, to vitamins B and A, respectively. The question arose are not the vitamins A and B essential for the normal life of higher animals in that they are the same as the *ergusia* and the *archusia*, respectively, or are essential in the formation of these substances. In a paper at the last meeting of this society, we showed that extracts rich in *archusia* act as vitamin B when fed to animals. It has been possible to test the relation between vitamin A and the *ergusia* in these experiments of Jorstad on coal tar. Coal tar absorbs the *ergusia* of the cell and causes them to show degeneration in the normally fed animal. It is toxic in small doses. In animals fed on a diet rich in vitamin A, Jorstad finds the cells do not degenerate and that these animals can withstand four times as much coal tar as animals fed on a diet poor in vitamin A.

It has thus been possible to come for the first time to some understanding of the nature of vitamins. Lipschitz and others have noticed that cancers do not develop uniformly in animals repeatedly painted with coal tar. What causes this individuality in the reactions of animals has been a question of discussion. It is possible as these experiments show that it is related to the *ergusia* content of the cells of the animals treated. Those rich in *ergusia* must suffer cancerous degeneration more readily than others in which the cells will degenerate rather than grow when drawn to drops of coal tar. The growth of the cells about the tar is a response to their becoming crowded together rather than any stimulation by the tar. The tar tends to decrease rather than increase their growth response. The cells rich in *ergusia* recover readily at the tar border, because this action of the tar is limited in each case to its

growth about the coal tar in this tissue is more marked.

In tissue with a high concentration of vitamin B, coal tar causes an attraction of cells from a wide area, but in no such proportions, as in the cases where the animal contains a high content of vitamin A. These cells show, however, a greater tendency to grow than in the animals with a low vitamin B. It brings about a set of conditions in the body similar to that seen in the tissue culture when *archusia* is added to the medium.

In tissues with a high content of vitamin A, the droplets of tar become dispersed into the tissue. Apparently this tissue is saturated with *ergusia*: the tar does not destroy the cells which it attracts to it. These cells remain active for a long time. In the mass of cells the *archusia* accumulates and they grow as a result. In tissue with a deficient amount of vitamin A, the cells degenerate as they migrate to the tar.

DISCUSSION

DR. MONTROSE T. BURROWS: In our earlier studies it has been possible to show that body cells have no special mechanism for migration. Their migratory activity is an adaptation of their growth reaction. It has been possible for us to analyze the mechanism of the growth reaction of body cells in the tissue culture.: They show a simplicity of structure not noted in other cells. The body cells cannot migrate under ordinary conditions into a water medium, but only into proteins and fats. Their movements are accomplished by their liberating a substance which is readily absorbed only by proteins and fats. This substance I have named the *ergusia*. It has strong affinities for the cell as well as proteins and fats in the environment. Mobile proteins and fats are drawn by it into the cell. The cell is drawn into fixed masses of proteins and toward larger masses of fat, which have a greater inertia than the cell. The first reaction in the cell is known as ingestion, while the latter is known as migration. This *ergusia* is not liberated by the cells under all conditions, but only when another substance, the *archusia*, reaches certain concentrations about the cells. The *archusia* is formed in the normal oxidative reaction of every cell. It is soluble in isotonic NaCl solution, in serum and in blood. The body cell has no means to retain this substance in it. Its concentration is determined always by the environment. The *arcusia* (S) in low concentrations has no effect. In slightly higher concentrations (S^2) it liberates the *ergusia*—the cell stores proteins and fats or migrates toward food. In higher concentration (S^3) the cell not only migrates and takes up food, but digests these proteins and fats and grows.

One Hundred and Eleventh Meeting, March 9, 1925

2. VITAMIN FEEDING AND COAL TAR STIMULATION.—By Dr. L. H. Jorstad.

From a study of the tissue culture, Burrows has shown that the growth of body cells depends on the formation and a certain high concentration of a primary oxidative product of body cells. This substance is not retained by the cell, but is readily washed away by the blood, serum or salt solution. For cells to grow independently, they must be crowded together in a small amount of medium, amply supplied with food and oxygen. As this primary oxidative product, the *archusia*, concentrates, the cells liberate another substance, the *ergusia*, which has strong affinities for fats and proteins. As the *ergusia* is liberated by the cell, small mobile particles of fat and proteins are drawn into the cells. Larger masses of these substances draw the cells to them. While the cells of the body can grow independently only when they can form and retain a large amount of their own *archusia*, they can be made to grow dependently by supplying them with *archusia* from other sources. In the normal organisms the growth is dependent. In cancer the cells are crowded and the circulation relatively reduced, the growth is independent. Anything that can primarily build such a tissue can form cancer. I showed in an earlier paper that drops of coal tar form such a tissue organization by dissolving the *ergusia* of the tissue cells and drawing them into a crowded mass about its periphery. Fresh drops of coal tar take so much *ergusia* away from the cells that many degenerate and the organism is often killed. I wondered whether the fat soluble vitamin A may not be important in forming the *ergusia* of the cell. I fed animals on a diet of deficient vitamin B, deficient vitamin A, high vitamin A, high vitamin B, and on a diet very rich in vitamin A. The animals fed on a diet rich in vitamin A withstand several times the lethal dose of coal tar for those fed on a diet poor in or which contains no vitamin A. The absence or presence of vitamin B has no effect on the toxicity of coal tar.

Experimental embryomata are more cellular and poorer in blood vessels than adult tissue. The *archusia* is present in greater quantity. The cellular

THE ACTION OF OILS IN THE PRODUCTION OF TUMORS

WITH A DEFINITION OF THE CAUSE OF CANCER *

MONTROSE T. BURROWS, M.D.

AND

CHARLES G. JOHNSTON, M.D.

ST. LOUIS

In 1906 B. Fischer introduced drops of a saturated solution of scharlach r in olive oil directly under the epidermis of the ears of rabbits. He notes that the epithelial cells grew down to surround these oil droplets. The histologic picture of this growth of the epithelial cells resembled epitheloid cancer in many cases, but these formations always ceased to proliferate and regressed after a time. Reinke introduced drops of ether into the eye of salamanders. A growth of cells was induced by the presence of this substance. He transplanted this new growth to the peritoneal cavity and found it continuing to proliferate for a time. White succeeded in inducing epithelial activity with drops of oleic acid, and Strober and Wacker with sudan III, indol and skatol. Benthin used several such materials, and Bullock and Rohdenberg and others have tried the effect of introducing scharlach r into various internal organs. In each instance these authors noted the same result: an encompassing of the oil with cells, certain grades of degeneration and proliferation, then a gradual regression of the newly formed tissue.

While there seemed little doubt from these experiments, as Wacker and others have pointed out, that certain lipoid solvents may excite epithelial growth, there was no evidence that they could excite true cancer.[1] On the other hand, it had long been known that certain workers exposed continuously to the action of soot (chimney sweeps [2]) and the action of other coal tar products are particularly predisposed to develop cancers of the skin, and that these individuals develop their cancers in localities of the skin where the soot or other substances are present frequently over a long period of time. Yamagiwa, appreciating

* From the Research Laboratories of the Barnard Free Skin and Cancer Hospital, and the Department of Surgery, Washington University Medical School.

1. Ewing, J.: Neoplastic Diseases, Ed. 2, Philadelphia, 1922.

2. Potts, P.: Surgical Observations, London, 1775.

the significance of the observations of B. Fischer and the relation of these observations to the development of cancer in coal tar workers, noticed that the difference between the experiments performed and the development of cancers in men exposed to lipoid solvents is that the animals studied received only a single dose while the men developing cancer were often irritated repeatedly by these substances for many years before their cancers appeared.

Yamagiwa and Ichikawa [3] then undertook the study of the effect of repeated applications of coal tar to certain restricted areas of the skin of the ears of rabbits. They found a piling up and proliferation of the epithelial cells of the skin after each application. Very large papillomas developed in some instances. In others the tissue not only suffered proliferation but also an associated degeneration and ulceration after each application of the tar. Eventually many of these tumors became cancers and metastasized. Since that time many workers have succeeded in producing carcinomas by repeatedly painting coal tar on certain areas of the skin of rats and mice (Woglom and Murray,[4] Fibiger,[5] Deelman,[6] Lipschitz [7] and others). Other similar substances, such as sudan III in olive oil and paraffin oil, have also given similar results. How these substances act to produce this disease has not, however, been fully explained. Clowes [8] suggested that these substances may act to initiate growth by dissolving the lipoid membrane of the cell. Bullock and Rohdenberg [9] thought that these substances acted to destroy the cells. They noted that many cells coming in contact with the tar degenerated. They considered that the growth followed this primary death of the cells. Champy [10] has outlined a similar theory. He concludes that the primary action of the tar is a destruction of cells. Other cells then grow to replace the defect from this loss of cells. The cells thus continuously stimulated to regenerate finally acquire the property of independent growth.

3. Yamagiwa, K., and Ichikawa, K.: Experimental Studies on the Pathogenesis of Cancer, J. Cancer Res. **3**:1 (Jan.) 1918.

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9. Bullock, F. D., and Rohdenberg, S. L.: A Study of the Scharlach R Reaction and of Allied Forms of Epithelial Proliferation, J. Med. Res. **33**:53, 1915.

10. Champy, C., and Vasilin, I.: Recherches sur le cancer experimental du goudron, Bull. du Cancer **12**:111, 1923.

Other authors have searched for specific substances in the tar. Several by-products of tar have been isolated and studied. Kennaway [11] states that isoprene causes cancers to develop more readily than coal tar. Other substances also have been isolated and studied. Kennaway, in one of his analyses, finds that the viscid tar of horizontal retorts is more active than the tar distilled in vertical ones, and that certain by-products of tar are more active than others.

It must be pointed out here that the more recent and older work on cancer has shown that it may not only be induced by coal tar and other lipoid solvents, but also by a number of different conditions and substances, such as roentgen rays, radium, certain animal parasites [12] and bacteria; that it may follow long standing chronic inflammation, atrophy from any cause, congenital tumor and defects, and probably in certain instances other injurious agents. In many of these studies, it has also been shown that these substances are not responsible for the eventual course of this disease but are important only in inducing it. Cancer is evidently not a disease to be classed with the infectious diseases [13] but is an active, independent growth of body cells. This primary growth is the result of some primary change either in the cell or in the tissue which may be induced by any one of the various substances or conditions mentioned above. When this change is once established, the cells then continue to grow indefinitely at the expense of the organism and independent of the causative agent. While it is true that Yamagiwa and Ichikawa [3] and later authors have shown quite definitely that coal tar repeatedly applied to areas of the skin will produce cancer, the cancers once induced have in each instance then proceeded independently of the tar. Many of these have been transplanted repeatedly to other animals and have continued to grow actively long after the tar had completely disappeared. The same is true of the cancers induced by animal parasites. Several years ago, Edwin Smith [14] isolated a bacterium, *B. tumefaciens*, from a cancerous growth in a plant. This organism is easily cultivated in a nutrient medium and will induce cancers when injected into other plants. Recently, Blumenthal, Auler and

11. Kennaway, E. L.: Cancer Producing Substances from Isoprene, J. Path. & Bacteriol. **27**:233 (July) 1924; On Cancer-Producing Tars and Tar-Fractions, J. Indust. Hygiene **5**:462 (April) 1924.

12. Fibiger, K.: On Spiroptera Carcinomata and Their Relation to True Malignant Tumors, with Remarks on Cancer Age, J. Cancer Res. **4**:367, 1919. Bullock, F. D., and Curtis, M. R.: The Experimental Production of Sarcoma of the Liver of Rats, Tr. New York Path. Soc. **20**:149, 1920.

13. Burrows, M. T.: Is Cancer a True Disease or Merely the Result of a Change in the Organization of the Organism? Radiology **4**:407, 1925.

14. Smith, E. F.: Studies on the Crown Gall of Plants, Its Relation to Human Cancer, J. Cancer Res. **1**:231 (April) 1916.

Paula Meyer [15] and Nuzum [16] have isolated several organisms from human cancers. One of the organisms isolated by the German authors resembled *B. tumefaciens*. Others were cocci. They found *B. tumefaciens* capable of inducing cancers in both plants and lower animals. The other organisms they have isolated have produced cancers in lower animals.

All malignant tumors do not contain such organisms. Even the most malignant tumors are often free from them. We have sought in vain to demonstrate bacteria in a rat sarcoma now being propagated in the laboratory. Jensen,[17] in studying the plant cancers induced by *B. tumefaciens*, has found that the bacteria are probably unimportant in doing more than inducing the cancer as coal tar, the roentgen ray and radium induce it. He found that these bacteria may disappear in these tumors after a time, although the tumor itself may be growing as actively as before. There is no evidence that the growth of the cancer induced by the action of roentgen ray and radium is due to the continuous action of the rays but to changes in the cells or tissues primarily induced by them. The same is true for the cancers of the stomach induced by the spiroptera [18] and the sarcomas noted by Bullock and Curtis [19] to form about the larva of the cat tapeworm in the liver of rats. The cancers proceed but these organisms always disappear.

The problem to be solved in the study of the action of coal tar and other lipoid solvents on the tissue is the nature of this change induced either in the cell or in the environment that allows the cells to grow independently. This problem cannot be solved through a study of the physical and chemical properties of these lipoid solvents alone, but will become understood only through a broadening of our knowledge of those general conditions which control cellular activity in the normal organism. The organism is a mass of cells. It has come about through a definitely regulated growth of the egg cell and the formation of the different types of cells. This organism matures and functions through a gradual change in the cells so that they use their energy for function rather than for growth. This organism maintains itself for a given period of time in that enough energy is used for growth to take care of the destruction suffered by the cells in their function.

There has never been any evidence to show, however, that the functioning body cells may not under the proper conditions utilize their

15. Blumenthal, Ferdinand; Auler, H., and Meyer, Paula: Ueber das Vorkommen Neoplastischer Bakterien in Menschlichen Krebsgeschwulsten, Ztschr. f. Krebsforsch. **21**:26, 1924.

16. Nuzum, J. W.: Experimental Primary Epithelioma from Micrococcus, Surg., Gynec. & Obst. **40**:343 (March) 1925.

17. Jensen, cited by Blumenthal (Footnote 15).

18. Fibiger: (Footnote 12, first reference).

19. Bullock and Curtis: (Footnote 12, second reference).

entire energy for growth. It is evident that in such a colony of cells as the body of man, in which the cells as a mass are using their energy for function, if one cell breaks away from the conditions that force it to function and acquires the property of independent growth, it must soon destroy its neighbors weakened by their functional burdens.

These recent researches have, therefore made the study of the action of oils in the production of cancer and the whole cancer problem one of the most fascinating and most hopeful for the development of broad biologic generalizations. The cancer problem as it is known today deals with those fundamental processes which regulate the normal growth, function and death of the organism.

Many of the earlier authors had already appreciated that cancer is an independent growth of body cells, a parasitism of one cell or a group of cells on their neighbors forced to work for the whole rather than acquire nutrition and use it for their own development. In attempting to explain it as such, certain of these writers thought that the control exercised by the whole over the growth and function of the cells was associated with permanent irreversible age changes in the physical and chemical structure of the cell. They looked, therefore, at the cancer cell as a cell that had become changed. It is a specially differentiated cell. The cancer cell arising from the skin epithelium differs from the skin in the same general manner as the skin epithelial cells differ from the muscle cells.

There was another group of authors among whom Ribbert [20] was preeminent, who took quite a different view. They looked at the active growth in cancer as the result of primary changes in the environment about the cells rather than in the cell itself. Ribbert based his argument on the fact that there is no evidence that the cells of the body lose their power to grow during development or in later life. That wounds heal readily in old persons and that the tissues of these old persons may regenerate prove this fact conclusively. The stopping of growth at maturity is the result of the acquired organization of the whole or the environment about the cells, and is not due to any irreversible age change in the cell.

In further proof for this deduction came the experiments of Driesch,[21] those of many other students of development, and the more careful analysis of the peculiarities of the cancer cells on which the adherents to the idea of a specially differentiated cancer cell clung. Driesch had noted that when a sea urchin gastrula is bisected, each half will regenerate into a complete sea urchin embryo and adult. These

20. Ribbert, H.: Das Karzinom des Menchen, sein Bau, sein Wachstum, seine Entstehung, Bonn, 1911.

21. Driesch, H.: Die Localization morphogenetischer Vorgange, Arch. f. Entwcklngsmechn. d. Organ. 8:35, 1899.

bisected halves do not begin to grow at once, however, but only after each has reformed by a shifting of cells into a perfect gastrula of one-half the normal size. The embryonal cells of the gastrula do not grow under all conditions. When the gastrula is bisected, they show only migration. Only when a special form is obtained in the whole does the growth of the cells again intervene. Again many students of cellular growth and cell division have shown that these acts may be initiated by outside means or by changing the environment, and that they are controlled definitely by the environment and not by the cell in the normal organism.[22]

The chief arguments that the cancer cell is different from the normal cell are that it has a different shape, it grows independently, it stains differently, its mitotic figures may be irregular and it may contain inclusions. Each one of these peculiarities has now been shown to be easily reproduced by simply changing the environment about any normal cell. The body cell is a fluid system that can be easily molded. Its shape is determined entirely by its outside contacts.[23] Any normal cell growing in the proper environment may take up foreign matter, may divide irregularly as cancer cells divide, and may stain as cancer cells stain. Wilson has shown that a substance that induces the unfertilized egg to develop normally will induce abnormal mitosis in eggs if allowed to act too long or in too great concentrations.[24]

While Ribbert and the schools proceeding and following him were unable to ascertain the nature of the environment suitable for cells to grow independently, the weight of evidence has always been with them in spite of the fact that a large majority of workers in this field have taken the other view. This flocking of the larger school to the standards of a different type of cells in cancer has not been, evidently, from an analysis of the facts at hand, but from the greater influence of men like Cohnheim and other leaders in pathology. The embryonal theory of cancer was apparently too fascinating. They refused to leave it and admit that cancer cells arising from the epidermis are nothing more than independently growing epidermal cells.

It remained, therefore, to find some means to isolate normal living body cells and study their habits in various kinds of environments. Reverdin had shown that small pieces of skin removed to a wounded surface grow and later attach themselves to the normal organism and aid

22. Wilson, E. B.: The Cell in Development and Heredity, Ed. 3, New York, p. 1029.

23. Burrows, M. T.: Energy Production and Transformation in Protoplasm as Seen Through a Study of the Mechanisms of Migration and Growth in Body Cells, Am. J. Anat., to be published.

24. Wilson, E. B.: Experimental Studies in Cytology, a Cytological Study of Artificial Parthenogenesis in Sea-Urchin Eggs, Arch. f. Entwcklngsmechn. d. Organ. **12**:529, 1901.

in covering the defect. Hertzler studied these skin grafts carefully to find that they take only when they are placed on a surface covered with a layer of clean fibrinous exudate. They grow into this exudate to make firm union with the body later.[25] In 1905 Harrison had noted that when the neural tube of young frog embryos is placed in a thin layer of lymph on the surface of a cover glass in a moist air chamber that the cells migrate and nerve fibrils grow from them into the clotted lymph. No actual growth of cells was noted, however, in these cultures by Harrison.[26]

The senior author, having the opportunity to work with Dr. Hertzler while he was performing his experiments on the healing of wounds in the peritoneum and skin grafting, appreciated at a latter date that the cells which grow first from the Reverdin skin grafts, grow quite independently of the body. Hertzler found that these grafts take only when the wound is covered by a layer of fibrin. In many such cases the grafts are separated from the underlying blood vessels by this fibrinous layer. Since substance diffuses poorly into such fibrinous layers, the cells migrating into the exudate from these fragments must be cut off largely from the body. These cells should grow readily, therefore, when removed to a layer of fibrinous exudate outside the body. In the summer of 1910, under a grant by the Rockefeller Institute, the senior author commenced to study the cultures of Harrison in his laboratory and attempted to develop a method for cultivating the tissues of higher animals. Blood plasma was first substituted for the lymph used by Harrison. In thin layers of plasma suspended from a cover glass into a small moist air chamber, the cells were found to grow readily and actively in many cultures of fragments of chick embryos.[27] These cells, it was interesting to note, did not grow in every culture of fragments of the same tissue placed in layers of the same medium, and where growth intervened the cells did not tend to arrange themselves into organ structures peculiar to their growth in the body, but they invaded the medium along the lines of least resistance, exactly as cancer cells grow and invade the organism. It seemed evident, therefore, that the secrets of the problem of cancer lay hidden in the conditions that allowed these cells to grow in this fashion in these cultures, or in the environment that the cultures

25. Hertzler, A. E.: The Peritoneum, Chapter V, **1**, 1919.

26. Harrison, R. G.: The Outgrowth of the Nerve Fiber as a Mode of Protoplasmic Movement, J. Exper. Zool. **9**:787, 1910.

27. Burrows, M. T.: Culture des Tissus d'embryon de Poulet et specialement cultures de nerfs de Poulet en dehors d l'organisme, Comp. rend. Soc. de biol. **2**:291, 1910; The Cultivation of Tissues of the Chick-Embryo Outside the Body, J. A. M. A. **55**:2057, 1910; The Growth of Tissues of the Chick-Embryo Outside the Animal Body, with Special Reference to the Nervous System, J. Exper. Zool. **10**:63, 1911.

imposed on them. One year later, this author then undertook in the Anatomical Laboratory of Cornell University Medical College, New York City, the problem of the conditions that allowed these cells to grow in the cultures. The medium chosen for this study was plasma prepared from the blood of healthy animals. The plasma was selected as medium because it represented a normal fluid existing everywhere in the normal organism. There was no evidence to show that cancer developed necessarily in an abnormal organism. In fact, transplanted cancer fragments grow best in young, healthy animals.

This study showed that in plasma this active independent growth of cells depends on three factors: (1) an ample supply of oxygen (which is about one third of the oxygen concentration of the air [28]); (2) a crowding together of the cells in a minimum amount of medium, and (3) a stagnation of this medium. These factors applied not only for the *growth of normal cells, but also for the growth of cancer cells in vitro* (animal and human cancer, carcinomas and sarcomas).[29]

Single cells carefully washed of tissue juices will not grow and often fail to show any reaction whatsoever when placed in a layer of pure plasma diluted with salt solution. On the other hand, if a salt solution extract of any actively growing tissue is added to the plasma, then single cells show immediate activity. They migrate, take up fats and proteins, digest these substances, grow actively and divide by mitosis.

In pure plasma, such an active growth will take place only about fragments of tissue. About these fragments, it is proportional to the age of the tissue, to the size of the fragment and cell density of the fragment and inversely proportional to the thickness of the layer of medium or the amount of absorbing medium about the cells. These proportions in relation to the size of the fragment and the thickness of the layer of medium hold only within limits of active diffusion of oxygen. These diffusion limits were measured and found to be from 1 to 2 mm. for most tissue fragments and from 0.5 to 0.7 mm. for clotted plasma or serum. About fragments of tissue larger than from 1 to 2 mm. in diameter, the growth is inhibited by a lack of oxygen to their central parts and toxic products liberated by the autolysis that results. Definite abnormal reactions are noted in cells in the deeper parts of layers of plasma thicker than 0.5 to 0.7 mm.

The relation of the size of the fragments and the thickness of the layer of medium was studied in fragments of tissue ranging from

28. Burrows, M. T.: The Oxygen Pressure Necessary for Tissue Activity, Am. J. Physiol. **43**:13 (April) 1917.

29. Burrows, M. T.: The Tissue Culture as a Physiological Method, Tr. Cong. Am. Phys. & Surg. **9**:77, 1913; Tissue Culture in Vitro, Tr. XVII Internat. Cong. Med., Gen. Path. and Path. Anat., London, p. 217, 1913; The Cultivation of Human Cancer Cells in Vitro, M. Rec. **86**:649, 1914.

1 mm. in diameter to a single cell, and in layers of plasma ranging from 0.2 to 0.7 mm. in thickness. In this study it was noted that while growth will obtain about fragments of a given tissue 1 mm. in diameter in a layer of medium 0.5 mm. thick, it will fail about a 0.5 mm. thick fragment of the same tissue in the same thickness of the layer of medium, but succeed about this smaller fragment (0.5 mm. thick fragment) in a thinner layer of plasma medium, 0.25 mm. thick.

Under these conditions of the proper thickness of the layer of medium and the proper size and cell density of the fragment, it is interesting to note that growth does not begin at once, but always after a given latent period. This latent period is shortest about the cellular fragment of actively growing tissues of young embryos and cancer. It increases in length about similar fragments of older and older embryos and is longest about the less cellular, nongrowing fragments of the adult.

As shown in another article [28] this difference in the latent period is not related to the age of the tissue in these cases, but to their immediate growth rate. It is much shorter for the cells of a piece of growing granulation tissue than for those of the normal connective tissue and shorter for the cells of an actively growing cancer than for those of a slower growing one.

It must be pointed out here that not only the growth of the cells is dependent on a proper supply of oxygen, stagnation, cell crowding and a proper minimum amount of medium, but all activity in these cells is dependent on these conditions. The cells in a cellular fragment of tissue first show migration, then growth and finally a complete disintegration or self-digestion.[30]. These various changes take place in regular sequence. Growth is only one phase of a general change induced in them by their environmental surrounding in the culture. Each of these changes begins in the cells of the center of the fragment. It then spreads from this point to involve more and more of the cells toward the periphery. The cells in a 1 mm. thick fragment of mesenchyme of a young embryo placed in a layer of plasma 0.5 mm. thick show all of these changes. As one decreases the size of such fragments, leaving other conditions unchanged, the amount of each of these changes not only decreases, but they begin to disappear in the reverse order in which they appeared about the larger fragments. In a culture of a medium size fragment self-digestion disappears except in the cells in the center of the fragment and growth becomes very limited. In still smaller fragments migration alone is observed. The cells in the latter cases do

30. This digestion of cells has been called self-digestion to distinguish it from autolysis. It is a digestion that takes place in the presence of oxygen and does not liberate toxic products. Autolysis, as the word is used, is a digestion taking place in the absence of oxygen. Toxic products are liberated in the autolysis of cells.

not disintegrate, but after becoming sufficiently separated they come to a state of complete inactivity. In this state they remain intact but inactive until destroyed by outside means. The cells in fragments of mesenchyme of older embryos and of the connective tissue of adults behave in the same manner. The cells in the sparsely cellular fragments show only migration and then inactivity. In the more cellular fragments growth and self-digestion intervene in proportion to the cell density of the fragments. The chief difference between these fragments of the older tissues and the more actively growing ones is that these changes take place after a much longer latent period.

From these observations it became evident that the life of these cells is something that is determined by their immediate surroundings. It is the result of a reaction between them and their environment. It has been taught that body cells are continuously active. Bayliss points out that there is no evidence for this view.[31]

These cells which had migrated from the fragment and come to rest apparently disproved it. Several such cultures have been kept in the presence of an ample supply of oxygen and in the incubator for as long as six months. After fourteen days the cells became inactive and remained so for the remainder of this entire period. They showed no changes in shape nor changes in their protoplasm. The fat droplets and others contained bodies ~~that~~ remained undisturbed in them during this time. At the end of this period these cells were found able to become active again. They migrated and grew when placed in drops of fresh plasma.[32]

The picture of life in these cells isolated from the body assumes, therefore, proportions of much greater simplicity than had been generally suspected from a study of them in the body. While previous students [33] of regeneration had appreciated that growth is regulated by factors other than food and oxygen, the nature of these other factors and their significance ~~seemed impossible~~ until we had found a method for isolating these cells and studying them under known and controllable conditions. Summing up the foregoing conditions necessary for their cellular activity in the culture, it became evident that they may signify that activity in these cells is dependent on the accumulation of some substance or substances formed by the cells but also readily diffusible into plasma. They also indicate that migration, growth and self-digestion may be merely the result of different concentrations of this substance or substances. Cells showing only migration and then complete inactivity had been seen only in culture of the more sparsely cellular fragments of tissue of older embryos and adults. Growth and

31. Bayliss, W. M.: The Principles of General Physiology, London, 1915.
32. Burrows: (Footnote 29, second reference, Fig. 4).
33. Morgan, T. H.: The Physiology of Regeneration, J. Exper. Zool. 3:457, 1906.

self-digestion are peculiar to the more cellular fragments of embryonic mesenchyme and cancer. When a large number of cells are crowded into a small amount of medium, all phenomena proceed. When few cells are placed in a relatively large amount of medium, the cells may become inactive after showing only migration.

An attempt was made, therefore, to ascertain whether such a substance or substances accumulated in these tissues during the latent period and accounted for the activities noted in the cells. Fresh fragments of actively growing tissue of embryos and cancer and slower growing tissues of older embryos and adults were extracted with an equal weight of isotonic sodium chlorid solution, and the extracts tested by placing equal parts with equal parts of plasma. The mixture was used as medium for isolated embryonic heart muscle and mesenchyme cells. Fragments of the same tissue left in a culture for from forty-eight to 128 hours were also extracted and the extracts tested in the same manner. In the presence of the extracts of the fresh tissues of the older embryos and adults, the isolated cells suffered little or no change, while the extracts of similar fragments left in the stagnant cultures for several days stimulated an active migration, growth and in some instances self-digestion in these isolated cells. Extracts of fresh tissue of the younger embryos and cancer stimulated immediate activity in these cells.

The cells of the more inactively growing tissues lying in the stagnant culture had evidently accumulated a definitely active substance. Extracts containing this active substance, S, were then carefully studied. In low dilutions (S^1), they were found to have no effect. In medium concentrations (S^2), they stimulated the cells to migrate and engorge themselves with particles of proteins and fat droplets. In high concentrations (S^3), these cells digested these proteins and fats, grew actively and divided by mitosis. In all higher concentrations (S^4), the cells suffered a self-digestion.

The picture of migration followed by growth and then self-digestion was only the result of a gradual accumulation of the S in the stagnant layers of plasma. A few cells had failed to grow in the thicker layers of medium because the proper concentration of the S could not be formed and retained by them. It is soluble in the salt solution and diffuses away into the plasmatic medium. A few cells cannot supply a large enough quantity of this substance to raise its concentration to the growing point in a large bulk of medium. About the less cellular fragments placed in a relatively large bulk of medium, the cells come quickly to rest because the diffusing S comes quickly to a concentration too little for any kind of cell activity. The cells cannot retain this substance. Its concentration is determined not only by the amount formed by the cells but also by the amount removed by the medium.

On account of these facts this substance or substances, *S*, has been named the *archusia,* the driving substance of the cell. It is evidently the energy for life in the cell. In studying its formation it was found that it is formed only in the presence of oxygen and certain food materials, not determined, but easily exhausted from a drop of plasma and renewed by adding fresh plasma or serum. Again, it was noticed that when it is present in the medium, the cells will react normally without oxygen until it is exhausted. They then suffer autolysis.[34] It is the only substance in the cell whose formation demands oxygen. In its presence digestion and synthesis proceed without oxygen.

The condition necessary for growth in the body cells is probably not different, therefore, from that noted by Wildiers for yeast. Single yeast cells will not grow in a large bulk of medium unless an extract of yeast is added, while a number of yeast cells added to the same quantity of medium begin to grow very actively after a latent period. Wildiers noted that this failure for the single cell to grow is due to the absence of a substance, *bios,* which is contained in the extracts of yeast and appears in the wine casks when a sufficient number of yeast cells are added.[35]

In the more careful analysis of this phenomenon discovered in body cells, the active substance, the archusia (*S*), has been found to be formed by the oxidation of food substances by the cell and shown to act to produce the manifestations of life in the cell, and to act according to its concentration to produce these different manifestations. It is evidently comparable, therefore, to the heat in the steam engine. The heat acts in the engine to produce work in proportion to its concentration. The archusia differs from the heat only in that it induces chemical changes in the cell rather than purely physical ones.

In the engine, it is well known that the work performed is as much dependent on the heat conserved for the water as the heat produced in the fire box. Take off the heat jacket of the boiler so that the heat may escape to the outside, the engine stops. It is interesting to note that the same holds for the cells. The body cell has lost or has failed to develop means to retain its energy, the archusia. This escapes from it or is not formed by it except when it is placed in a stagnant environment rich in food and oxygen, and in association with other cells also forming it. In recent years a great amount of study has been devoted to determining the amount of oxygen adsorbed in the body and the carbon dioxid and water eliminated. It is interesting that these studies have yielded little of immediate practical importance. From the

34. Burrows, M. T.: The Reserve Energy of Actively Growing Embryonic Tissues, Proc. Soc. Exper. Biol. & Med. **18**:133, 1921.

35. Wildiers, E.: Nouvelle substance indispensible an developments de la levure, La Cellule **18**:311, 1901.

studies cited above, it becomes evident that the activity of the body is not alone dependent on the energy produced but also on the energy conserved.

FUNCTION AND DEVELOPMENT OF DIFFERENT TYPES OF CELLS OF BODY

In the first cultures of chick embryos, active rhythmic contraction of the heart muscle cells was also observed in the fragments and whole hearts transplanted to the cultures.[37] It was interesting to note that these heart muscle cells which had been contracting in the fragment lost this ability to contract as they migrated from the fragment into the medium of most cultures. In the medium they assumed the shape, grew and divided like simple mesenchyme or sarcoma cells. It became evident, therefore, at this early time that whether these cells are to grow, divide and invade the medium or contract rhythmically depends probably on their immediate surroundings.[36] In these cultures of embryonic tissue, it was interesting to note, however, that their tendency was to revert from a functional to a growing state. Only the cells in the more central regions of the fragments continued to contract, while those in the periphery stopped this act and invaded the medium as fast as the cells outside them moved away.

The cells in fragments of embryonic striated muscle from the limbs and back also contract rhythmically in the cultures. The contractions are exactly like those of the heart muscle. These muscle cells in contact with the medium also quickly revert to cells indistinguishable from the simple mesenchyme or sarcoma cells. Under proper conditions they may grow actively and divide by mitosis, or they may migrate as long spindle shaped cells or spread from the tissue as an open syncytial network.

While in the great majority of cultures, there is no tendency for these cells which migrate from the fragment to revert and develop function again, in an article[37] published in 1912, one cell and an open syncytium were noted to undergo this change. Since that time eight more such isolated contracting cells and contracting syncytia of cells have been observed in eight cultures out of several thousand studied with this and other ends in view. These cells when cut from the circulation of the body and placed in the stagnant culture accumulate archusia about them and thus tend always to revert to a state of growth rather than function.

36: Burrows, M. T.: An Attempted Analysis of Growth, Anat. Rec. **9**, Paper 11, 1915; The Tissue Culture in Cancer, Tr. Second Pan. Am. Sc. Cong., Washington, Section 8, Part 2, p. 494, 1915.

37. Burrows, M. T.: Rhythmische Kontraktionen der isolierten Hertzmuskelzelle ausserhalb des Organismus, München. med. Wchnschr., No. 27, p. 1, 1912; Science **36**:90, 1912.

In the cultures where one places a fragment of embryonic muscle tissue from 0.5 to 1 mm. thick, the central parts of these fragments suffer a self-digestion [38] from an overabundance of the archusia. A liquid substance is liberated by this digestion. This liquid flows out over the surface of the layer of medium to form a tough film. This film is not only rich in food material but also in archusia. The heart muscle cells entering it flatten out to take an otherwise spindle or irregular shape, grow actively and divide by mitosis. The fragment is also rich in accumulated archusia at this state.[23]

Noting the latter facts, it had become of interest to see if the environment played any rôle in determining this rhythm in such isolated rhythmically contracting cells. While it is true that much has been written about the mechanism of muscular contraction, nothing is known except certain isolated facts:

1. Berstein noted several years ago that the energy of muscular contraction has a negative rather than a positive temperature coefficient. Only surface energy can have a negative coefficient in muscle.

2. Fletcher and Hopkins had noted that lactic acid is liberated at the contraction phase and that oxygen is absorbed only during relaxation.

3. Electrical responses to contraction and the heat changes in muscle have also been carefully measured.

It was interesting in observing these contracting heart muscle cells to note that they occupied a peculiar position in the medium. They were not at the surface of the medium nor in the clot but stretched through a serum cavity between the fragment or the surface film and the ends of the bands of fibrin. One end of these cells was in contact with an area rich in archusia; the other end was in contact with bands of fibrin extending into the open medium. In this medium the archusia diffuses readily. This end in contact with the fibrin must have a very low archusia value.

These cells had gained this position through adhering to the clot, which had loosened from other parts of the side of the fragment and contracted away from it. They had thus become stretched between the fragment and the clot. In other cultures isolated cells were dragged out by the same means so that they became stretched between the surface film and the ends of fibrin fibrilli in the clot.

In studying the action of archusia on the cells, it was found that it acts to liberate another substance, the *ergusia*. This substance decreases the surface tension of cells. Such a decrease in surface tension is associated with electrical changes. This decrease in surface tension causes the

38. Burrows, M. T.; Burns, J. E., and Suzuki, Y.: Studies on the Growth of Cells: The Cultivation of Bladder and Prostatic Tumors Outside the Body, J. Urol. **1**:3 (Feb.) 1917.

cell to stretch in the direction of its lowest surface tension. In these cells this decrease in surface tension can take place only at the end of the cell in contact with the fragment or the surface film. Under the influence of this decrease in surface tension at one end, these cells must suffer one of three kind of changes: (1) a breaking loose from the fibrin; (2) a tearing of the cell in the middle, or (3) a breaking down of the surface tension lowering substance or the substance that adsorbs it. In the great majority of cultures in which fibrin contracts as described above, the cell quickly breaks loose from the clot. None of them break in the middle. In a few out of the several thousand rhythmicity resulted. These cells contract as the result of a periodic reduction in their surface tension followed in each instance by a breakdown of this surface tension lowering substance or the substance adsorbing it. Lactic acid is liberated. Lactic acid increases the surface tension of the cell or makes it shorten. The ergusia reforming decreases the surface tension or makes the cell lengthen. Such a breakdown is associated with an electric current passing from one end of the cell to the other. It will result always when any simple system like these cells is polarized as the contracting heart muscle cells were found to be polarized.[39]

On several occasions such contracting cells were removed from this position to the serum. In the serum they became spherical in shape and inactive. The same cells removed to the surface film stretch out to take a flattened spindle or irregular shape, grow and divide and look like sarcoma cells. Others were placed in the fibrin outside. They remained inactive or stretched out along the fibrin fibrilli and became inactive.[30]

It was thus possible to show absolutely that function and growth in these heart muscle cells are determined by the environment and not by the cell. They are reversible phenomena. The change of a cell from the state of active function in the normal organism to the growth observed in cancer must be a normal reaction of the cell to a changing environment. Ribbert's contention had thus been realized.

While it was thus possible by means of the tissue culture to transform a functioning cell to a growing cell and vice versa, it has not been possible to transform a connective tissue cell into a muscle cell or an epithelial cell into any other type of cell. These cell types are determined by some stable internal mechanism in the cell. This fact one might have readily deduced from the study of cancer, however. There is no evidence that a cancer cell is other than the growing connective tissue or epithelial cell from which it arose. Teratomas develop only from egg cells. The egg cell can give rise only to multiple cell types. A

39. Burrows, M. T.: A Note on the Mechanism of Heart Muscle Contraction, Am. J. Physiol. 45:556, 1917.

growing smooth muscle or striated muscle cell resembles growing connective tissue cells.

There is no evidence, as many recent authors have attempted to show, that the cancer cell is a specially differentiated cell, nor is there any evidence in the whole of embryology or experimental biology that such a cell may arise. On the other hand, it can be fully proved by the tissue culture that any cell may grow if given the proper surroundings and this growth may be independent. These surroundings are simply those which allow a certain high concentration of their archusia to accumulate about them. This archusia may be supplied from without or by the cells themselves when they are crowded into sufficiently narrow stagnant confines. Under the latter conditions, the growth of body cells becomes wholly independent. This is cancer. When supplied from other sources, their growth becomes dependent on the supply.

Function on the other hand comes into existence when the cell is so placed that the archusia remains in concentration only at one end. The type of function is determined by the more fundamental constitution of the cells in question. Connective tissue cells and epithelial cells stretched as the heart and striated muscle cells were stretched (described above), do not contract rhythmically. Smooth muscle cells so stretched contract rhythmically, but their contractions are not like the heart muscle and striated muscle cells, short and rapid, but are slow. They are exactly like the contraction peculiar to smooth muscle in the body.

In the body all functional cells have this arrangement. The nerve fibers are stretched between the dense brain tissue and an end organ. The gland cells are in contact with an active blood circulation on one side and a stagnant gland tube on the other. The archusia is washed away by the blood stream from their outer ends and accumulates at their inner ends. Functioning glands and nerves show electrical changes as muscle shows these changes.[31] While their function is different from that of the muscle cell, it is determined by the same organization and is evidently the result of an explosive breakdown of a polarized system. It differs from the muscle because of a different physical chemical make-up in the epithelial and other cells.

In the culture function had been forced on the heart muscle cells through a contraction of the clot away from the fragment. In the body the cells are forced to function through the development of intercellular tissue and blood vessels. In further proof for these deductions, it is noted that epithelial cells from glands of the body revert in the cultures to broad sheets of migrating and growing cells. Champy [40] found that if connective tissue is added to such culture the epithelial

40. Champy, C.: La presence d'un tissue antagoniste maintient la differentiation d'un tissu cultive en debois de l'organisme (Note preliminaire), Comp. rend. Soc. de biol. 76:30, 1914.

cells are forced to form tubular structures again. Drew [41] has confirmed these observations of Champy.

CONDITIONS REGULATING GROWTH IN THE BODY

In the light of these facts, growth and function in the body take on quite different aspects. Function is something peculiar to an active circulation in properly arranged blood vessels. Growth is something peculiar to stagnant crowded cell areas where the circulation is poor. In this regard body cells are not different from any of the simple unicellular organisms. It is in the stagnant pool and not in the running stream that such life abounds. Wildiers had found that the growth of yeast depends on conditions identical with those outlined above for the growth of body cells. In 1923 experiments with a culture chamber which allowed the tissue to be washed continuously with a stream of serum were described. In these experiments it was found that washing prevents the growth of heart muscle cells but accelerates and makes their rhythmical contractions regular and forcible.[42]

In the development of the animal body, it is interesting to note that growth is most active at the beginning. It wanes and is replaced by function as the blood vascular system develops. At maturity growth ceases except as it is necessary for repair, regeneration and in certain parts, such as, the nails, hair, sex glands and bone marrow.

Areas undergoing repair and various of the more permanently growing regions and cancerous tissues have been studied. In the wound, growth does not intervene alone at its edge but throughout the wide area of tissue behind its edge, which is suffering from the inflammatory slowing of the circulation. This growth behind the edge of the wound is proportional to the amount of congestion and slowing of the circulation. In the wound the greatest growth takes place in the stagnant wound areas where the blood vessels have been completely destroyed.[43]

The distal end of the growing nail bed has a greatly reduced circulation. This area is supplied by a single layer of large sinusoids. These sinusoids are larger than the vessels which fill and empty them. The bone marrow has the same sluggish sinusoidal circulation. The growth in the sex gland is inside the tubules and follicles far removed from the blood vessels. Cancer is a densely cellular tissue supplied by irregular tortuous vessels few in number per unit cell area or by large dilated

41. Drew, A. H.: Growth and Differentiation in Tissue Cultures, Brit. J. Exper. Path. **4**:46 (April) 1923.

42. Burrows, M. T.: Studies on Cancer, 4 papers, Proc. Soc. Exper. Biol. & Med. **21**:94, 1923.

43. Burrows, M. T.: Studies on Wound Healing: I, "First Intention" Healing of Open Wounds and the Nature of the Growth Stimulus in the Wound and Cancer, J. M. Res. **44**:615 (Sept.) 1924.

sinusoids. These vessels are being continuously destroyed in its more central parts. Hemorrhage and necrosis are the result.[44]

In the light of these facts it became evident, therefore, that cancer may be nothing more than the result of any substance or condition that can primarily build in the organism a dense mass of cells having a relatively poor blood supply. Cancer is only such a tissue organization. It is not a primary change in the cell.

The proof for this deduction lay in showing that such an organization once induced can reproduce itself and destroy the organism and that the substance or conditions, such as coal tar, which induce it, act only to produce such an organization.

In a previous article,[43] one of us has already discussed and given proof that such an organization once established preys on and destroys the surrounding less crowded and more vascular normal tissue. All these normally arranged cells are attacked alike, including the endothelial cells of the blood vessels. A mass of cells thus sufficiently crowded and stagnant not only reproduces itself through a growth of its cells but also the environment suitable for a continuation of this growth by its continuous destruction of its own vascular supply and that of the tissues about it. This destruction of the blood vessels is greatest in the tumor. The central parts of these tumors suffer necrosis. The peripheral parts receiving sufficient oxygen continue to grow actively.

This attaching of a less dense mass of cells by another denser mass was first noted in a study of the behavior of fragments of normal skin of embryos of various ages in the cultures. In the early embryonic life, the epidermis is a single or a double layer of cells. The underlying connective tissue or mesenchyme is a densely cellular layer. As development proceeds in the embryo, the epidermis gradually becomes a thick cellular layer while the mesenchyme cells cease to multiply at an early time. This layer expands rather by the laying down of intercellular substances, the connective tissue fibrils. When this laying down of fibrils between the mesenchyme cells takes place, they become widely separated so that the number of cells per unit area in this layer becomes only a fraction of their original value. When fragments of the skin of the younger embryos are cut from their circulation and placed in the cultures, the mesenchyme cells grow most actively and destroy and devour the protoplasm of the cells of the less cellular epithelial layer. About fragments of older and older embryos, the activity of the mesenchyme wanes in the cultures and the epithelial layer becomes more and more active to dominate completely about the fragments of older

44. Burrows (Footnotes 13, 42 and 43); Factors Regulating Cellular Growth and Their Importance in the Explanation of Cancer, South. M. J. 17:233 (April) 1924.

embryos, as the mesenchyme had dominated in the earlier period. These active epithelial cells then destroy the cells in the less densely cellular connective tissue area. This growth of densely cellular layers can be greatly facilitated by adding embryonic extracts to the plasma.

Epithelial cells, as Fischer [45] has shown, do not grow readily in the cultures unless these embryonic extracts are added to the medium. Barta [46] has carefully studied the behavior of epithelial cells in fragments of the ureter of young rats. The ureters of rats are small. One millimeter fragments of the whole cross section can thus be placed in the culture without destroying the oxygen supply to all parts of this fragment. In a medium containing embryonic extracts, he found that the epithelial cells grew actively to invade the connective tissue and fat of the adventitia in a manner typical of true cancer. These growing cells reduced the muscle, blood vessels and connective tissue to a hyaline mass and destroyed the fat tissue. Their invading epithelial cells contained mitoses, irregular, large multinuclear cells, and were in every way identical with cancer.[46]

Maximow has found the same phenomena in fragments of breast planted in a medium containing extracts of bone marrow.[47] Burrows had noted a sarcomatous overgrowth of the mesenchyme in embryonic fragments placed in pure plasma and the phenomena greatly exaggerated in a culture of these tissues into which an extract of embryos or cancer tissue is added. A few of these fragments transplanted into the subcutaneous tissue of an adult rat developed into true sarcoma.[48]

Burrows has also found that this dominance of a more cellular and less vascular layer over a more vascular and less cellular layer occurs in wounds as well as in the tissue culture and in cancers. When the wound is open and suffering a congestion and slowing of its circulation, the granulation tissue grows actively in areas removed from the originally more densely cellular epidermis. As the epidermis moves in over the wound and the circulation is reestablished in the granulating area, growth ceases in this area, but continues in the more densely cellular epithelial layer until the granulations are reduced to a hyaline mass. The cells and blood vessels are destroyed by this growth of the epithelial layer, which continues until its blood supply is thus reduced and atrophy intervenes.[48]

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45. Fischer, Albert: A Three Months Old Strain of Epithelium, J. Exper. Med. **35**:367 (March) 1922.

46. Barta, E.: Experimental Histological Studies, I. Some Factors Regulating the Morphology of Tissue (Ureter in Vitro), Anat. Rec. **29**:33, 1924.

47. Maximow, A.: Tissue Culture of Mammary Gland, Anat. Rec. **27**:210, 1924.

48. Burrows, M. T.: The Experimental Production of Malignant Ulcers in Rats, J. Missouri State M. A. **20**:145, 1923.

Such scars may become the site of cancers. It has also been found that the failure for the wound to become cancerous in every case is due to the fact that the epithelial layer under ordinary conditions does not become thick enough to overpower the resistance of the body outside. If this layer is stimulated to increase in thickness through the addition of growth stimulating substances to it, cancer may then intervene."

THE ACTION OF COAL TAR AND OTHER LIPOID SOLVENTS IN THE PRODUCTION OF CANCER

Before attempting an investigation of the action of coal tar and other lipoid substances in the production of cancer, it seemed necessary first to study more carefully the general metabolism of fat by body cells. While many authors had studied the heat produced in fat metabolism and the various by-products into which fat may be changed, no analysis of the ingestion of fat and fat bodies by the cell had been undertaken.

In the early part of this article it has been shown that under the influence of a medium concentration of the archusia (S_2) the cell takes up fat, but does not digest it. Under the influence of higher concentrations of the archusia (S_3), the fat and proteins so taken up are digested and growth intervenes. How this fat is taken into the cell was then investigated by the senior author.

In this analysis it was noted that body cells, unlike many of the unicellular organisms, have no distinct mechanism for migration. They are simple fluids. They can migrate only into substances containing fats and proteins which have greater inertia than themselves. If the fats and proteins are in suspension and are mobile, they are drawn to the cells. If these same substances are fixed or large and have a greater inertia than the cell, the cells are drawn to them. The mechanism for migration in body cells is the mechanism for taking these necessary food substances into themselves. Migration is merely an adaptation of the growth reaction of these cells as function is an adaptation of the same reaction. These cells migrate or draw food into themselves by liberating a substance that has strong affinities for the cell as well as proteins and fat of the medium. This substance or substances has been named the *ergusia*, the laboring substance of the cell to distinguish it from the *archusia*, the driving substance of the cell.

In the plasma cultures the cells do not liberate the ergusia under all conditions, but only when the archusia accumulates to a certain concentration (S_2), as has been pointed out above. In the presence of this concentration of the archusia, the cell liberates the ergusia[49] which is adsorbed by the clot and fat in the clot. If the clot is held firmly through attachments to the glass surface on which it is placed, the cells are drawn out into it and the fragments spread and flatten out. If the

49. Credit for the words archusia and ergusia is due to Prof. Thomas S. Duncan of the departments of Greek and Latin, Washington University, St. Louis.

clot is not attached, it is drawn in mass to the fragment. In the same manner, small, mobile fat droplets are drawn into the cell. Larger, less mobile masses of fats draw the cells to them.

In studying the ergusia in the different types of cells it is interesting to note that it differs in different cells. It has only one definite common property: it is adsorbed readily only by proteins and fats. The ergusia of the connective tissue coagulates blood to fibrin and serum. The ergusia of the epithelial cells has the same property, but these cells differ from the connective tissue cells in that they may later dissolve the fibrin formed. The ergusia of the leukocytes and lymphocytes does not form fibrin to any great extent. Their ergusia is readily adsorbed, however, by both the fibrinogen and fats.

In proof that the cells migrate and acquire their food by this means, it was not only found that their movements are often unassociated with any changes in their contour (they migrate without ameboid movements) or evidence of other mechanical mechanisms, but also that they are exactly proportional to the liberation of this substance as such liberation is identified by the action of the ergusia of the connective tissue and epithelial cells on a plasma clot, and through the proof that the fat taken up by the cells becomes saturated with this ergusia.[23]

In a short time after fats are eaten by animals their blood contains a large amount of this fat. It occurs in the blood in the form of suspended droplets. The plasma, prepared from such blood, is rich in fat. If a fragment of connective tissue is placed in a drop of this plasma, the cells become filled with these fat droplets. The fat moves rapidly out of the plasma into the cells. The fat has no coagulating effect on the clot. It prevents its coagulation rather than stimulates it. This fat after it is taken up by the cells acts differently. If the fat droplets are squeezed from these cells and placed in fresh plasma, they coagulate the plasma, stick to the fibrin fibrils thus formed and assume spindle shape quite the same as the connective tissue cells. When placed near other connective tissue cells, they repel these cells or are repelled and driven away from the cell exactly as one connective tissue cell repels another in the culture. These last phenomena continue, however, only until the ergusia which they have adsorbed is lost to the clot. Then they are adsorbed again by the cells when brought in contact with them.

In other experiments, it was found that this taking up of the ergusia follows the laws of adsorption or solution. There is a definite saturation point for ergusia in proteins and fats. These cells can migrate into these substances or draw them into themselves only until the concentration of the ergusia in the particles is balanced with that in the cell. Then all activity ceases.[36]

In the light of these experiments, it became evident, therefore, that if one places in the tissue any viscid, more or less immobile and non-

digestible substance that has strong affinities for the ergusia it must draw the surrounding cells to it and away from their intercellular substances and blood vessels. It can thus disrupt the normal tissue organization up to the time it becomes saturated with this substance. This action accumulates the scattered cells of the tissue in dense stagnant masses about the absorbent and thus builds an organization in which the cells can accumulate their archusia and grow. It builds a nonvascular cancerous organization.

ACTION OF COAL TAR

Having established these facts, Jorstad[50] in this laboratory then undertook the study of the action of coal tar in the production of cancer. Jorstad injected small quantities, from 1 to a few cubic centimeters, of crude coal tar into the subcutaneous tissue of rats and also into embryonic tissue that had been cut into small fragments and injected under the skin of animals. These masses of coal tar were in some cases left for a long time and then removed. Others were removed at regular intervals of twenty-four hours and longer. In a few instances the coal tar was injected just beneath the epidermis so that the reaction of the epithelial cells could also be observed. In most instances it was injected into the subcutaneous tissue far removed from the epithelial layer.

The change induced by the drops of tar is a rapid migration of the cells of the tissue to their edges. In a few cases the tar broke up into a number of smaller drops. In most cases, however, it remained together as one large drop. These cells accumulating from wide areas in the tissue formed a collar around the drops of tar. The first cells to react to the drop underwent a granular degeneration in most of the experiments. The later cells remained intact. The cells chiefly attracted were the connective tissue cells and the endothelial cells of the capillaries. The capillaries themselves, therefore, disappeared through this movement and separation of their endothelial lining cells. The intercellular materials were little disturbed except that they showed a hyalin-like degeneration and became stripped of the fibroblasts, which normally lie scattered among them.

The action of the single drop of coal tar was limited in this capacity. Its action was most rapid at first. Then this activity ceased slowly, to stop in the course of a few days, as any substance that dissolves substances from a medium ceases as the distribution constant is satisfied between it and the medium.

In the sparsely cellular subcutaneous tissue, but few cells were thus collected by a single drop of coal tar. In the more cellular mesenchyme of embryos, the tar accumulated many more cells. This was also true

50. Jorstad, L. H.: A Study of the Behavior of Coal Tar on the Tissue, Proc. Soc. Exper. Biol. & Med. **21**:67, 1923; The Behavior of Coal Tar in Embryonic and Adult Tissues, J. Cancer Res., to be published.

when the tar was placed in contact with the more densely cellular epithelial layer. In the adult connective tissue, growth was never observed in the cells collected by the tar. In the more cellular mesenchyme of embryos in which a greater number of cells were collected, these masses became larger and more densely cellular. In these larger masses growth and division figures were seen. Against the epithelial layer of the skin the tar collects more cells, and in these denser masses of epithelial cells growth and division figures are always seen. This growth often leads to the formation of pearls and the picture of precancerous lesions. In the epithelial layer of embryonic tissues, typical cancerous lesions made their appearance.

The action of a single drop of coal tar is limited. It does not act to stimulate the cells to grow but only to draw them to it and accumulate them in dense masses about it. If these cell masses become sufficiently dense and large enough, growth may then later intervene as the archusia of these cells accumulates about them. To produce such large masses many applications of tar are generally necessary. With a single injection this is never accomplished in the animal under ordinary conditions. The cells collected soon suffer regression and hyaline changes, therefore. Eventually a hyaline scar surrounds the drops of tar. In a few instances after a time the drops of tar saturated with ergusia begin to migrate. They enter the veins and migrate to distant organs in the same manner as fat droplets squeezed from body cells migrate from fragments or other cells into the less saturated parts of the medium of the culture. The blood contains little or none of the ergusia peculiar to the fixed tissue cells. It acts as a solvent for this substance and attracts the tar.

These observations indicate, therefore, that the action of coal tar is not chemical, but purely physical. It acts merely by dissolving a lipoid soluble substance of the cells. It is possible that in the fractional distillation of coal tar one will find a substance having a more efficient solvent action, but it is not likely that any important growth stimulating substances will be found, as many of the English authors have supposed. Any oil having a similar solvent action must act accordingly.

ACTION OF PARAFFIN AND PARAFFIN OILS

To prove the latter deductions more definitely, it became of interest to see how other oils act in this regard. Previous studies have shown that paraffin and paraffin oil applied frequently to a point on the skin will induce cancerous growths, as coal tar induces them. Mook and Wander [51] noted that single injections of paraffin into the subcutaneous tissue of men lead to the production of tumors. They

51. Mook, W. H., and Wander, W. S.: Camphor Oil Tumors, Arch. Dermat. & Syph. 1:304 (March) 1920.

found these tumors composed of drops of the oil enclosed in a hyaline, fibrous capsule. These tumors are similar, therefore, to the tumors that Jorstad found in animals injected with single drops of coal tar.

The paraffin in the cases studied by Mook and Wander had been injected into patients as a vehicle for camphor and other drugs. This discovery that this oil forms tumors led to the discontinuing of its use as a vehicle and the substitution of Mazola, or corn oil.

It is well known that corn oil has no immediate food value other than the fat it contains. It is largely free from vitamin. One of us (C. G. J.), in performing experiments with the Allen-Doisy hormone,[52] noted that this oil also produces tumors. The Allen-Doisy hormone was dissolved in this oil and given hypodermically. It thus became of interest for us to study the action of pure corn oil when injected into the subcutaneous tissue. Previous authors had noted that many oils of the food when injected subcutaneously are not absorbed, but no one had studied the action of these oils on the tissues.[53]

THE ACTION OF MAZOLA, OR CORN OIL

Thirty-nine rats, one monkey and one dog were used for these experiments. From 1 to 5 c.c. of the pure sterile corn oil was injected into the subcutaneous tissue of each animal. The oil was injected in each instance so that it formed one large oil droplet. This mass broke up quickly into numerous drops of various sizes. These drops remained unabsorbed. Each drop then became firmly encapsulated so that a multiple cystic tumor was formed, similar in each case to the one removed and photographed (Fig. 1). The nature of the changes that this oil induces in the tissue was studied by removing such tumors at regular intervals of one, two, three and one-half and seven days, after two and four weeks, and after two, three, five and seven months.

PROTOCOLS

EXPERIMENT 1.—Two cubic centimeters of sterile Mazola oil was injected into the subcutaneous tissue of four rats. After twenty-four hours the oil tumors were removed. They consisted of a large number of small and larger cysts each enclosed in a grayish capsule, which varied from 1 to 3 mm. in thickness. One of these tumors was fixed in formaldehyd, the other three were fixed in Zenker's fluid; a part of the formaldehyd fixed tissue was cut with the freezing microtome and stained for fat. Other parts and the Zenker fixed tissue were dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and

52. Allen, Edgar; Francis, B. F.; Robertson, L. L.; Colgate, C. E.; Johnston, C. G.; Doisy, E. A.; Kountz, W. B., and Gibson, H. V.: The Hormone of the Ovarian Follicle; Its Localization and Action in Test Animals, and Additional Points Bearing upon the Internal Secretion of the Ovary, Am. J. Anat. **34**:133 (Sept.) 1924; The Extraction and Some Properties of an Ovarian Hormone, J. Biol. Chem. **61**:711 (Oct.) 1924.

53. Henderson, Yandell; and Crofutt, E. F.: Observations on the Fate of Oils Injected Subcutaneously, Am. J. Physiol. **14**:193, 1905.

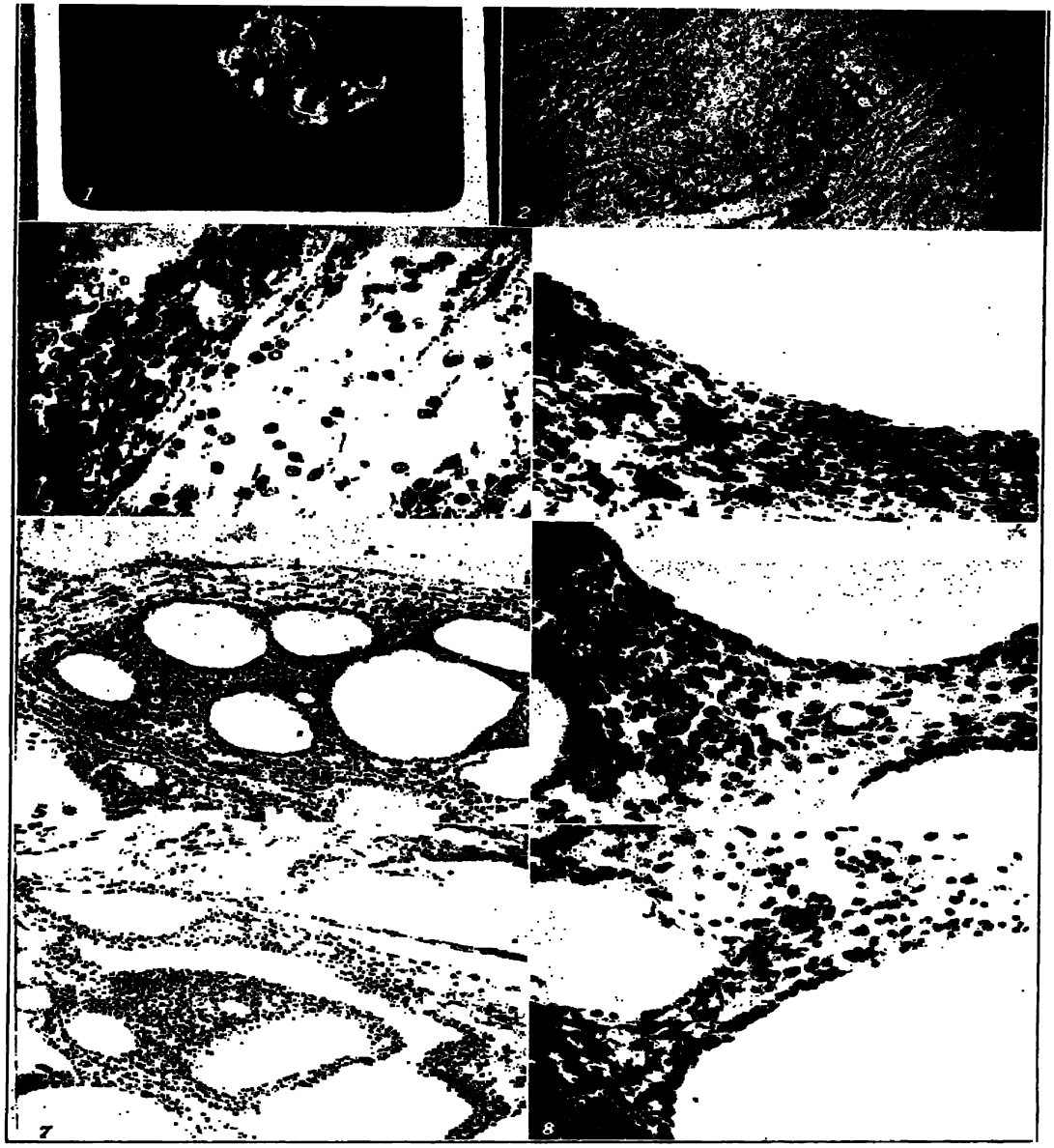

Fig. 1.—Carefully dissected 9 day old oil tumor from subcutaneous tissue of rat.

Fig. 2.—Low power photomicrograph of section of oil tumor 24 hours old.

Fig. 3.—High power photomicrograph of section of oil tumor shown in Figure 2.

Fig. 4.—Photomicrograph of section of 24 hour old oil tumor that has been fixed in formaldehyd, cut with the freezing microtome, stained with sudan III and hematoxylin and cleared in glycerin. The oil has fallen out of the cavity. The small droplets are seen between the cells in the wall.

Fig. 5.—Low power photomicrograph of section of 48 hour old oil tumor of rat.

Fig. 6.—High power photomicrograph of section of oil tumor shown in Figure 5.

Fig. 7.—Low power photomicrograph of section of 7 day old oil tumor of rat.

Fig. 8.—High power photomicrograph of section of oil tumor shown in Figure 7.

eosin. At this early stage, one saw the oil carefully cut off from the surrounding fibrils of the fibrous tissue by a layer of cells varying from 1 to several cells in thickness. The whole of the surrounding tissue was filled with cells evidently migrating toward the oil. The immediately adjacent cellular capsule was ragged; the cells were not closely matted together in it. A few had penetrated a short distance into the oil (Fig. 2).

In studying more carefully the various parts of these sections, it was interesting to note that the extent of this cellular reaction varied in the different tissues into which the oil had been placed. It was much greater about the drops placed in the more cellular fibrous tissue than in the fat tissue.

The types of cells responding in most areas were chiefly large spherical or ovoid cells having a well formed vesicular nucleus. Among these larger round cells in most areas one found also a scattering of small mononuclear cells, a few polymorphonuclear neutrophils and a larger number of polymorphonuclear eosinophil cells (Fig. 3). The relative number of the latter cells also varied with the regions into which the drops were placed. Among the more numerous blood vessels of the subcutaneous tissue, one saw a larger proportional number of lymphocytes and leukocytes than in the less vascular fibrous tissue and fat.

With this movement of cells toward these drops of fat, tiny droplets of fat were continuously breaking off from the larger one and moving out into the tissue. This moving out of small fat drops was in proportion to the movement of the cells toward the larger drops. These small fat droplets could be readily brought into the field of vision at all periods of the development of these tumors by staining the frozen formaldehyd fixed sections with sudan III and hematoxylin and clearing the section with glycerin (Fig. 4).

At first we thought that these large mononuclear cells with a vesicular nucleus were wandering cells of some sort or another, as many other authors have considered them. It soon became evident, however, that this was not true. As seen in the section of the tumor taken at a later period, these cells were fibroblasts that had loosened from their attachment to their intercellular fibrils and endothelial cells, which had separated themselves from the capillaries through the action of the oil on them.

By means of the tissue culture, it was possible to determine that the shape of the fibroblasts is not determined by any internal organization peculiar to them, but to chemical and physical reactions that they induce in the environment about themselves. They are simple fluid systems. When connective tissue cells are placed in body fluids containing fibrinogen, they coagulate the fibrinogen to fibrin and stick to the side of the fibrin fibrils. They are fastened to the fibrin by the ergusia. These fibrin fibrils, as Hertzler [25] has shown, become the intercellular fibrils of the connective tissue. The oil dissolving this ergusia not only loosens these cells from their attachment to the fibrils, causing them to round off as any drop of fluid rounds off under these conditions, but it also draws them to it. At later periods after the oil droplets have become saturated with the ergusia, the cells slowly assume other shapes according to the shape of the proteins which they coagulate in their environment.[23]

EXPERIMENT 2.—Four rats were injected the same as in Experiment 1, and the tissue removed was studied and treated in the same manner. The difference between this experiment and the first one was that the tumors were removed after forty-eight hours. At this period the cells had ceased to migrate to any extent from the tissue outside. The cells at the edge of the oil droplets

had become more closely packed together into a layer, several cells in thickness (Fig. 5). The inner layer of cells had in many places formed a continuous layer of cuboidal or flattened cells much like endothelial cells. Outside this inner cuboidal layer, the large mononuclears were stretching out more on less in many places to spindle and irregular shaped cells. These changes were suffered only by the large round cells with vesicular nuclei. The lymphocytes and leukocytes remained scattered among them as before (Fig. 6).

EXPERIMENT 3.—In this experiment one rat was injected in the same manner as the others. The tumor was removed after three and one-half days. It showed no important differences in its morphology from those removed after forty-eight hours.

EXPERIMENT 4.—Four rats were injected as in the other experiments. The tumors were removed after seven days. At this period the picture had changed little from that of the earlier periods, except for a further organization of the cells about the fragment into a definite connective tissue (Fig. 7). Most of the large mononuclear cells were stretching out to take a spindle shape along the forming intercellular fibrils, or had alined themselves into a layer of flattened or cuboidal cells about the oil droplets (Fig. 8). The fine intercellular fibrils noted in the specimen removed at forty-eight hours (Fig. 6) had now become more prominent; a true intercellular fibrous tissue had made its appearance in many places (Fig. 9). Most of the polymorphonuclear and small lymphocytes still remained scattered among these cells. They had taken no part apparently in the actual formation of the capsule, however.

EXPERIMENT 5.—Twenty-two rats were injected as in the other experiments. The tumors were removed from these rats under ether at fourteen days, one month, two, three and seven months. The tumors were fixed either in formaldehyd or Zenker's fluid and stained with hematoxylin and eosin or hematoxylin and sudan III. In most of the specimens the changes were a gradual increase in the organization and then a hyalinization of the capsule about the oil droplets. The oil remained unchanged during this later period (Figs. 10, 11 and 12).

While the oil remained unchanged in practically all these cases, in one rat it was apparently absorbed. This absorption took place slowly, and as the oil disappeared the circumference of the cellular wall either shortened or the tumor collapsed. It was interesting to notice that the cells in the capsule of such cases of absorption did not tend to lay down intercellular substances but remained closely packed together much the same as they were at the beginning. They also showed some evidence of growth in that their nuclei contained more chromatin and their cytoplasm stained more deeply (Fig. 13). Mitosis also was observed in one of these cases. There was never any evidence of the oil stimulating the cells to grow in any of the other cases. The oil attracted the surrounding cells of the tissue to it, but it did not excite growth in these cells. It acted rather to cause them to degenerate. Evidences of growth were seen in these sections only when the oil was placed in a sufficiently cellular tissue so that a large number of cells became massed at its periphery. When the number of cells became large enough, as in Figure 15, then one saw sharply staining cells. Growth in these cases is evidently a secondary phenomenon and the result of the massing of the cells together. It was secondary to the original action of the oil which pulled them into this mass.

EXPERIMENT 6.—One dog was injected in the same manner as the rats. The tumor was removed after three weeks. It was interesting that in this animal the oil was being slowly adsorbed as in the case of the one rat described above (Fig. 14). The cells about the absorbed oil droplet showed evidences of stimulation. The conditions leading to growth and the absorption of the oil in these cases were not determined.

EXPERIMENT 7.—One monkey was injected with 5 c.c. of Mazola oil. The tumor was fixed in formaldehyd after nine days. The oil had broken up into numerous droplets. Each of these tumors was enclosed in a cellular fibrous

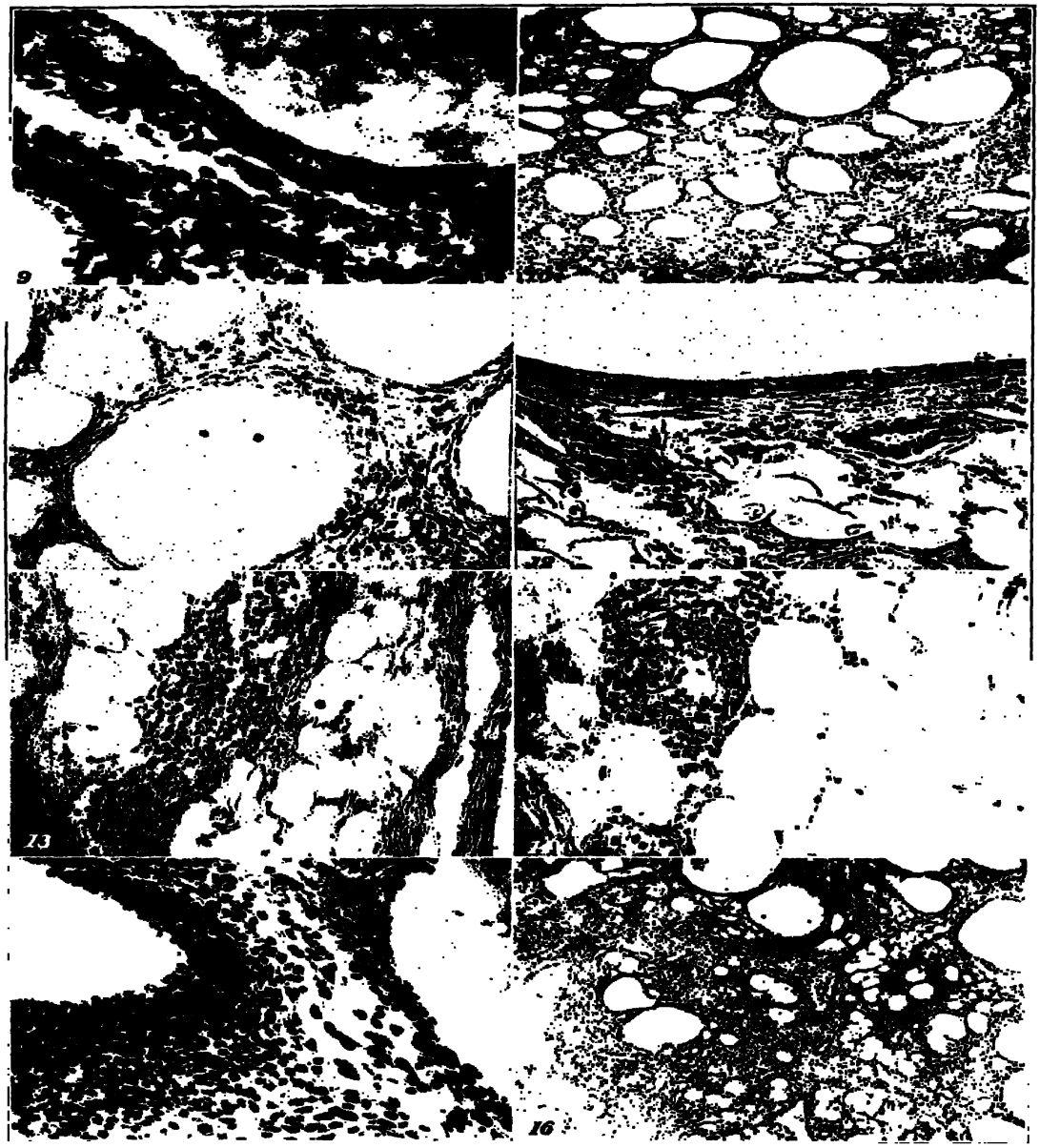

Fig. 9.—High power photomicrograph of another part of section of oil tumor shown in Figures 7 and 8.

Fig. 10.—Low power photomicrograph of section of 22 day old oil tumor of rat.

Fig. 11.—Low power photomicrograph of section of 7 months old oil tumor of rat.

Fig. 12.—High power photomicrograph of section of 24 day old oil tumor of rat.

Fig. 13.—Mass of cells marking site of oil cyst in which the oil was absorbed.

Fig. 14.—Section of wall of oil tumor from subcutaneous tissue of dog; the oil has been absorbed and the circumference of the tumor has reduced accordingly.

Fig. 15.—Dense mass of cells at edge of oil droplet showing evidence of growth in 7 day old oil tumor of rat.

Fig. 16.—Low power photomicrograph of section of 9 day old oil tumor from subcutaneous tissue of monkey.

capsule like that seen in the majority of the rats. None of the oil had been absorbed (Fig. 16).

EXPERIMENT 8.—Two rats were injected with pure Mazola oil that had not been sterilized. The tumors were removed after seven days. The picture in these cases was different. The oil was surrounded by a mass of fibrinous exudate filled with polymorphonuclear cells. This typical inflammatory reaction was quite different from the picture of the simpler migration of wandering and fixed tissue cells to the sides of the sterile oil droplets.

Mook and Wander noted giant cells in the hyaline capsule surrounding droplets of paraffin oil. They considered that the oil acted as a foreign body. They called these giant cells foreign body giant cells. The tumors they classified as sarcoids.

It must be pointed out that paraffin oil applied frequently to a given place in the skin will lead to cancerous production. What Mook and Wander studied were single injections. The oil that had been injected in many of their cases was not pure paraffin oil, but oil containing camphor. This substance excited a true inflammation. There was an exudation of fluid and fibrinogen from the blood vessels, and when necrosis intervened polymorphonuclear cells made their appearance.[54] The fibrinogen and fluid formed a thick mass about the irritant or diluted it. The fibrinogen clotted to a gel. As stated above, oxygen diffused readily into such clots no more than from 0.5 to 0.7 mm. The cells become abnormal beyond this distance from the source of oxygen. Barta [55] has recently studied these abnormalities and finds that cells situated a distance greater than 0.7 mm. from the air surface of the layer of plasma medium of a culture change into reticular, epithelioid and giant cells. These cells are not in any way concerned with foreign bodies but owe their formation entirely to a reduction in their oxygen supply. Fibroblasts, certain wandering cells and other tissue cells will show these changes. The giant cells noted by Mook and Wander can only be indicative of such an inflammatory process. This same inflammatory process we noted about drops of unsterile corn oil. The sterilized oil acts only to dissolve the ergusia of the cells. Since the ergusia has strong affinities for the oil as well as the cells, the oil is thus drawn toward the cells and the cells to the oil. The larger oil droplets become encapsulated by drawing the cells to them from the tissue and blood vessels in proportion to the original number of these cells in these localities. Smaller droplets of fat are seen among the cells. They are drawn from the larger drops by the same process that the cells are drawn to these larger drops.

54. Burrows, M. T.: Neuritis of the Cranial Nerves in Lethargic Encephalitis and the Differential Anatomic Diagnosis Between It and Acute Poliomyelitis, Arch. Int. Med. **36**:477 (Oct.) 1920.

55. Barta, E.: Experimental Histological Studies, II, Giant Cell Formation and Fat Metabolism in Relation to Oxidation (Lymph Nodes in Vitro), to be published.

While many previous authors have looked to the discovery of some hormone or growth stimulating substance in coal tar and other lipoids capable by long use of inducing cancer, others have appreciated that such is probably not the case. They had noted that the oil acts to cause the cell to degenerate rather than grow (Bullock and Rohdenberg [9] and Champy [10]). What stimulated the eventual proliferation of these cells they could not determine. The tissue culture has answered this question. It is the result of the crowding of cells induced by the oil attracting the cells of the tissue to it away from their intercellular substances and blood vessels.

It is now well established that to induce cancer with coal tar and other lipoid solvents these substances must be applied frequently over a long period of time. The reason for this is clearly shown above. A single drop of oil becomes quickly saturated with the ergusia, or lipoid substance, of the cell. It can pull only a few cells to it. With each addition of fresh oil to the same place, more cells are pulled in until finally there is built a stagnant mass of cells of sufficient size to overcome the growth energy of the surrounding tissue.

COMMENT

Previous work on cancer has shown that it may be induced by a variety of conditions and substances: It follows on the site of chronic inflammation; it may arise in many benign congenital and other types of benign tumors and congenital defects; it may be induced by coal tar, other lipoid solvents, roentgen rays, radium, bacteria and certain animal parasites. While many of these substances and conditions were shown capable of inducing cancer, it was also noticed that they are unimportant in the eventual course of this disease. Cancer is an independent growth of body cells which may be induced by any one of these conditions and substances but, once established, then proceeds independently of them. The first important problem to be solved in cancer is the conditions allowing such an independent growth of the cells.

This problem was investigated by one of us (M. T. B.) by means of the tissue culture. It was found that single cells cannot grow in a simple body medium, such as blood plasma. Growth can intervene in this medium only about densely cellular fragments and it proceeds about these fragments only when they are placed in a very small stagnant quantity of this medium, which is well supplied with oxygen. This growth depends on the accumulation and maintenance of a certain concentration of a primary product of the cells about them. This product is formed by them through the oxidation of food materials. It is soluble in the blood, in serum and in isotonic sodium chlorid

solution. This substance or substances has been called the archusia, or the laboring substance, of the cell.

The body cell is a drop of fluid cytoplasm in which floats a fluid nucleus. It also contains fat droplets, centrosomes, mitochondria and other formed bodies floating in its cytoplasm. These cells have no organization other than centrosomes (active only in cell division) for transforming their energy into work. These cells can induce reactions between certain food substances and oxygen with the formation of the archusia. The archusia acts on the cell in proportion to its concentration. In low concentrations (S_1) it has no effect. In a medium concentration (S_2) it acts to liberate another substance from the cell's protoplasm, the ergusia. The ergusia is a lipoid soluble substance readily adsorbed by fats and proteins. Small mobile particles of proteins and fats are drawn into the cell as this substance is liberated into a medium in which it can diffuse. Larger masses of proteins and fats draw the cells to them. The cell grows by this ability to take up these proteins and fats. Its migration is the result of the same reaction and occurs when the cell meets masses of proteins and fats having a greater inertia than itself. The ergusia is not the same in the different cells. It is highly selective in its action on proteins but not on fats. All cells are attracted by fats. The ergusia of the connective tissue cells coagulate fibrinogen to fibrin while the ergusia of the leukocytes and lymphocytes does not. The different cells in the body differ from each other in their chemical make-up. They grow always to reproduce themselves. This specificity in their growth may be readily referred to the specificity of their ergusia and the specific selection of food substances by it.

In high concentrations the archusia (S_3) causes the proteins and fats to be digested; the cell grows and divides by mitosis. In all higher concentrations (S_4) the cell is digested and destroyed.

The archusia is apparently the same in all cells. The archusia extracted from one cell sets up the reactions mentioned above in other cells. None of these cells except possibly the striated adult muscle fibers can retain their archusia. For them to grow independently they must be crowded, therefore, into a small stagnant area in which their archusia cannot escape from about them. Since the archusia is not specific, these same cells can be made to grow dependently, however, by supplying archusia from the outside. In such cases their growth must be limited always to the supply, and wholly under the control of the outside forces supplying this substance.

In the light of these facts, it became evident that cancer may be none other than the result of a primary reorganization of tissue in the body. There was no evidence that the independently growing cells of the culture had changed primarily. The change leading to their growth

was in the environment. Applying these principles to the study of growth as it is observed in the body, it became evident that all growing tissues of the normal organism are cellular and have a stagnant circulation and that this stagnation and cell crowding reaches its greatest development in cancer. The question therefore arose, Is cancer other than the result of the building of a dense mass of cells free from blood vessels? The proof of this deduction lay in showing that such a mass once built will reproduce itself and that the substances and conditions that lead to cancer act only to build such a tissue organization.

It was found by means of the culture and a study of wounds that any dense, stagnant mass of tissue may not only grow but also destroy readily any less dense and stagnant tissue. Not only the surrounding tissue cells but also blood vessels suffer in this destruction. Such a mass once established can thus continuously reproduce itself through a growth of its cells and the continuous destruction of its blood supply.

The action of coal tar and corn oil in the production of cancer have been investigated. It has been found that they have no stimulating action on the growth of the cells, but act only to disrupt the normal organization of the tissue and build a densely cellular tissue relatively or wholly free from blood vessels and intercellular substances. These oils act to collect these cells merely by dissolving the ergusia, the lipoid-like substance of the cell. The cells are drawn to the edge of the drop merely through this adsorption by the oil of their ergusia. The ergusia liberates the energy for the migration of the cell, through its strong affinities not only for the cells but also for the oil. It thus decreases the surface tension of the cell in the presence of the oil.

A single drop of coal tar, corn oil or paraffin is limited in this capacity. It soon becomes saturated with the ergusia. It cannot draw a sufficient number of cells or form a sufficiently large mass of these cells for them to overcome the resistance of the outside and thus take food to themselves. With each new addition of the oil, more and more cells are drawn to the drop until a cancer is built.

We have not studied the action of roentgen rays, radium and animal parasites in the laboratory. It must be pointed out here, however, that the only tapeworm larva known to induce cancer is the cysticercus of the cat tapeworm. This larva produces changes in the tissues of the host which are different from those produced by the larvae of tapeworms, which do not induce cancer. It induces a densely cellular tissue capsule about it. This capsule is a dense mass of fibroblasts, while that induced by the echinococcus, a noncancerous producing parasite, is a hyaline, fibrous one containing very few cells. Wolbach,[56] in 1909, had shown that the roentgen ray acts on the tissue to reduce

56. Wolbach, S. B.: The Pathological Histology of Chronic X-Ray Dermatitis and Early X-Ray Carcinoma, J. M. Res. 21:415, 1909.

the blood vessels and change them. It acts, therefore, also to build a cancerous organization.

Bacteria are now being studied carefully. They act differently from the tar to produce, however, the same nonvascular cellular organization in the tissues. Smith had noticed that *Bacillus tumefaciens* when introduced into the tissues of a plant induces the cells to proliferate actively. In studying the action of this organism in the animal, we find it causes the cells with which it comes into contact to proliferate without the formation of normal body structures, such as the blood vessels and intercellular substances. It acts to produce by proliferation a local dense mass of cells that is identical to the masses formed by the drops of oils through collecting the scattered cells of the tissues about them. Extracts of embryo and Berkefeld filtrates of malignant tumors, which are rich in archusia, act in the same manner to stimulate the tissue cells to proliferate. Drops of oil act to disrupt the normal organization of the tissue and build one peculiar for cancer in that they are able to attract the cells from the tissue to them (Fig. 17). The bacteria produce the same organization by stimulating the cells that lie between the blood vessels to proliferate and thus to form a crowded cellular mass (Figs. 18 and 19). The blood vessels do not grow to any extent because the stimulus is continuously washed away by them and cannot concentrate on their lining endothelial cells.

The latter experiments on the action of bacteria are interesting in that they show that certain bacteria can liberate growth stimulating substances not unlike those liberated by body cells.

In the further study of the properties of tissue cells, it has been shown that these simple active fluid systems when polarized or placed in an environment in which the archusia can concentrate only at one of their ends, suffer a rhythmic explosive breakdown of either the ergusia liberated at the one end where the archusia is high or the substance adsorbing it in the environment. These breakdowns are expressed in muscle by contraction, in the gland cells by secretion, in the nerves by conduction. Function is the result of such a polarization. In the body this polarization is induced by the interspersion of blood vessels between double columns of cells. In the culture function is produced by placing the same conditions about the cells. The blood vessels bathing one end of the cell remove the archusia formed at this end of the cell. It accumulates at the other end of the cell away from the blood stream.

In the light of the foregoing observations, cancer maintains when the cells are crowded together in an area having a greatly reduced blood supply. Function is a property of a rich blood supply in properly arranged blood vessels. It is something which takes place at the expense of the growth reaction of the cells. It reaches its greatest development in an environment that must inhibit growth completely

unless the cells are supplied with archusia by other means. Function is something which has been imposed on the cell by outside forces and not something which the cells will tend to develop at their own initiative. The question confronted us, What is the nature of these outside forces?

As has been noted, single cells isolated in a drop of plasma and groups of cells washed with a stream of serum cannot grow. The same

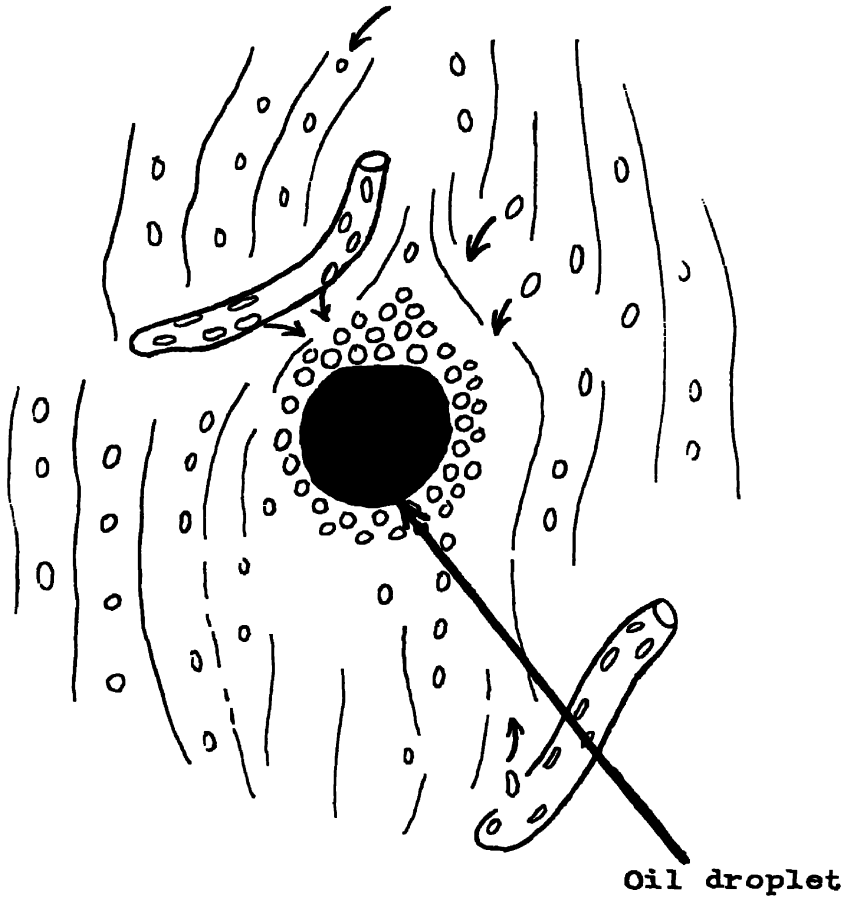

Fig. 17.—Manner in which a drop of oil attracts cells when placed in the tissue; it disrupts the normal organization of the tissue and builds a cancerous organization.

cells can be made to grow by adding archusia to these mediums. As stated above, such growth is not independent but dependent on the source of the archusia. In the animal organism there is no evidence to show that the cells lose their power to grow at any time. The prevention of an active, independent growth of these cells and function in the normal organism is wholly dependent on the organization of the normal organism, the arrangement of its blood vessels and cells.

In other studies in this laboratory, it has been found that the cells of the normal body do not grow independently at any time from the egg to the death of the adult. At no place are they crowded sufficiently and their environment sufficiently stagnant to allow them to grow without an outside source of archusia. In cancer conditions are different; the organization about the cells has changed to one in which the cells are crowded sufficiently and their relative circulation sufficiently reduced for them to form enough archusia and retain it in a concentration ample for them to grow independent of any outside source.

In the light of these facts it becomes evident, therefore, that growth in the body must be at all times dependent on a source of stimulus from

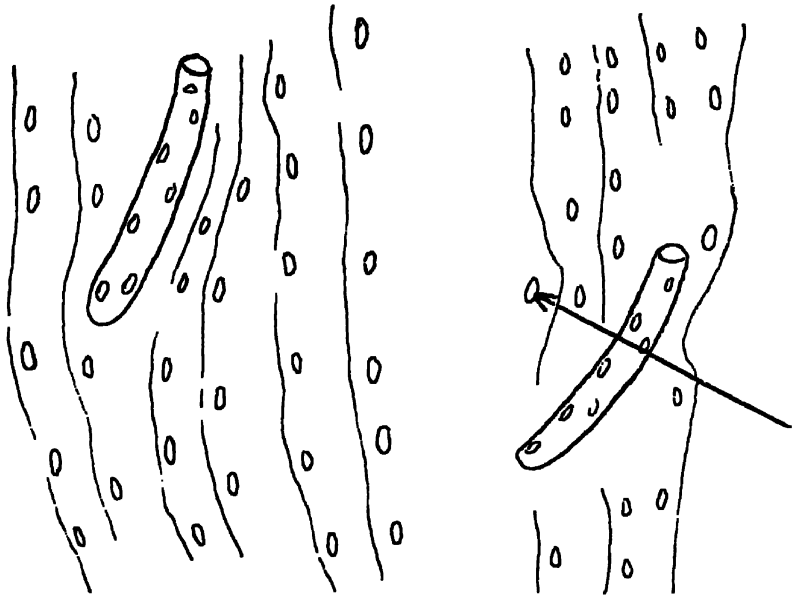

Fig. 18.—Site of introduction of tumor forming micro-organism or extract of an actively growing tissue.

the outside: the mother, the yolk of the egg or the food. It is a well known fact that one cannot exist on proteins, fats and carbohydrates alone. Other accessory food substances are necessary. These have been termed vitamins. It has been shown above that when *B. tumefaciens* is introduced into the tissues it stimulates the cells to proliferate. They grow to form a densely cellular nonvascular mass. The same is true for extracts of embryos or any other actively growing tissue. Under normal conditions these substances cannot gain entrance to the body except through the food. In the food they must enter the blood vessels first and then gain access to the cells. Under such conditions they will not act to build a cancerous organization. The blood vessels must

suffer the greatest stimulation. They must grow, therefore, in excess of the cell between them and form a vascular rather than a nonvascular tissue.

In the light of these facts, it became of interest for us to study the action of *B. tumefaciens* and the extracts of embryos when injected and when fed to the animal. One of us had already noted that extracts of actively growing embroyos when injected into the skin stimulate an

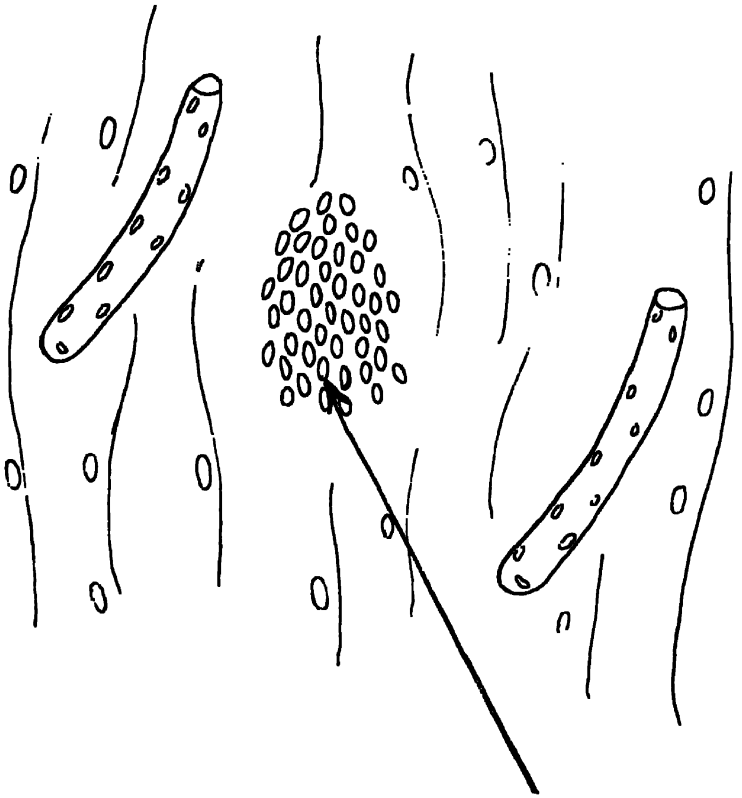

Fig. 19.—Result of introduction of tumor forming micro-organism or extract of an actively growing tissue into the tissues. The cell proliferates without the formation of intercellular substances and blood vessels. These bacteria or tissue extracts by stimulating the cells to proliferate accomplish the same result as oil in attracting the scattered cells of the tissue.

active growth of cells and cancers.[48] We are now investigating the effect on the growth of young rats of feeding *B. tumefaciens* and extracts of embryos.[57]. Two day old and 5 day old cultures of *B. tumefaciens* (Smith) were fed to rats in a diet free from vitamin B and in a diet free from vitamin A. The rats fed on 2 day old cultures in a

57. Burrows, M. T.: Studies to Determine the Biological Significance of the Vitamins, Proc. Soc. Exper. Biol. & Med. **22**:241, 1925.

diet containing vitamin A (butter) grew as well as those fed on the same diet containing autolyzed yeast instead of the bacillus. The rats fed on the 5 day old culture grew as actively. Extracts of embryos that had been shown to be rich in the archusia have also been fed. These extracts act only as vitamin B substitute. In other experiments we have sought for stimulating substances in the glands of internal secretion. The studies on the ovary have now been completed. The Allen-Doisy hormone extracted from the follicular fluid of the ovary is found to be rich in an active growth stimulus. Whether this stimulus acts as substitute for the vitamins we have not determined.

From these observations on the effect of feeding these substances on the growth of the animal, it becomes evident, therefore, that the control of body growth, its form and function must depend on three factors: (1) a complex egg cell capable of giving rise by division to multiple cell types; (2) an absence of the power of the cells of the body to retain their archusia, and (3) an active supply of archusia from an external source aided probably by certain glands of internal secretion.

It has been possible for us to show in the laboratory that when a stimulus is introduced into the tissue it stimulates an active growth of cells and a cancerous organization. When fed it produces a normal vascular tissue. When placed in the tissue it stimulates the cells to grow without organization. From the food it enters the blood vessels first, and stimulates them to grow more than the intervening tissue cells. A vascular-functioning tissue is thus formed.

The bodies of higher animals must be, therefore, the result of an evolutionary development. Their function and normal growth is not a property peculiar to their cells, except in an environment rich in growth stimulating substance, the archusia or something similar. This substance forcing its way in from the outside builds the vascular system. Function is not a normal act of the cells, but something imposed on them by the body organization thus controlled from without. The bodies of higher animals survive, grow and function only through this outside aid. Such stimulants are formed probably only by living cells. The body exists through preying chiefly on lower nonfunctioning growing forms. While the glands of internal secretion must aid in the directing of these stimuli, the chief source of these stimuli is probably from without.

In the cancerous state the cell alone reaches its full independence. In further proof for this deduction, it is interesting to note that Cramer [58] has found that the growth of transplanted tumors of rats is not influenced by the absence or presence of vitamins. We have

58. Cramer, W.: Dietary Deficiencies and the Growth of Cancer, Eighth Scientific Rep. Imper. Cancer Res. Fund, London, p. 17.

repeated and confirmed these experiments.[60] In cancer the cells are able to live an existence independent of specific substances in the diet. In the crowded, stagnant environment of cancer they can conserve their energy, the archusia, and build their parts from simpler substances.

The cancer cell is not primarily different from the normal cell. The primary change in cancer is a reorganization of the tissue so as to produce a densely cellular mass having a relatively poor circulation. While it has been shown from the work of Nuzum,[16] Blumenthal, Auler and Meyer [15] that a certain number of cancers of man undoubtedly owe their origin to bacteria, it must be clearly emphasized here that this does not indicate that cancer is infectious. Cancer may be produced by any one of a number of conditions and substances that primarily build a dense mass of cells in the organism in such a manner that the circulation is so reduced that this mass receives at its periphery an ample oxygen supply, but is otherwise stagnant. The conclusions drawn by Oxner [60] and Nuzum [16] are not based on the facts at hand and are thus wholly uncalled for and may do harm.[61] Blumenthal, Auler and Meyer have carried their work further than Nuzum. They found it impossible to draw such conclusions.[62] Cancer is the normal means of the cell to survive when unaided from without. It must always be the normal outcome of a certain number of aging organisms.

The difference between the cancerous tissue and the normal is the difference between the greater conservation of growth energy in the cancer than in the body outside. Our immediate hope for the medical treatment of cancer does not lie in an ability to equalize the energy in the body over that in the cancer, but in finding some means to overwhelm the growing cancer cells. If we raise the amount of archusia in the blood to that in the cancer, the growth of the cancer must cease, but function cannot maintain under these conditions. Function depends on the absence of, or a very low, archusia at the end of the cell in contact with the blood stream and a high value at the opposite end. The amount of function is the difference in these energy values. When we demand increased function, the circulation increases. When we eat a big dinner and load our blood with growth stimulus from without, we become

59. Burrows, M. T., and Jorstad, L. H.: Unpublished notes.

60. Oxner, A. J.: Cancer Infection, Surg., Gynec. & Obst. **40**:336, 1925.

61. Soper, S. A.: The Application of Facts and Opinions Resulting from Laboratory Experiments to the Practical Work of Cancer Control, Surg., Gynec. & Obst. **40**:334, 1925.

62. The same evidently applies to the recent work of Gye and Barnard reported in the lay press and in J. A. M. A. **85**:368 (Aug. 1) 1925, at the time this proof was being corrected. The Rous chicken sarcoma has long been suspected as being due to a bacterium. This is not true of many of the sarcomas and carcinomas of rats and other animals. Gye and Barnard will undoubtedly find that extracts of any actively growing tissue, many bacteria and other substances capable of stimulating cells will induce cancer if properly introduced (Footnote 48).

dull and drowsy. After a day of active blood circulation, we become exhausted, our blood pressure drops, we sleep. The cells must be allowed to grow, to recuperate their loss of substance through function.

We have found, however, that when the archusia is raised in the medium of a tissue culture to a certain high concentration (S_4) the cells are digested. Cancer cells and actively growing embryonic cells can be made to suffer such digestion by adding to the medium containing them only one-tenth of the amount of archusia capable of stimulating growth in fresh normal adult tissues. Whether this can be used for treatment is yet to be investigated. It is possible that other more simple means will be found to destroy the cancer without destroying normal cell. The means to discover such substances or condition will probably be found in a more careful analysis of roentgen rays and other substances inducing cancer and in more study of the details of cellular growth and function.

CONCLUSIONS

1. The tissue culture has given us an entirely new idea of the structure and activity of body cells. Our earlier ideas had been gleaned entirely from a study of complexly organized unicellular organisms and a study of the morphology of body cells. The tissue culture has allowed us to study for the first time the mechanism of growth and function in these cellular elements. They are one of the few cells in nature which have a simplicity of structure sufficient to make such an analysis.

2. Cancer is an independent growth of these cells. It may be induced by any one of a number of conditions and substances. Once established, it proceeds independently of these causative agents.

3. An active independent growth of body cells depends on a crowding of the cells together in a stagnant area or an area having a relatively poor blood supply. Cancer has this kind of an organization.

4. It has been found that such an organization can reproduce itself through the fact that it destroys through its growth the normal tissue and blood vessels about it.

5. The action of drops of corn oil and coal tar on the tissue has been investigated. It has been found that they act to build a dense, stagnant mass of cells by drawing the tissue cells to them and away from their intercellular substances and blood vessels.

6. The bacteria that induce cancer act in the same manner to build a densely cellular stagnant mass of cells, but by a different means. They induce such a mass of cells to form by stimulating the cells of the tissue to proliferate without forming to any extent intercellular substances and blood vessels.

7. These factors of stagnation and cell crowding become important for growth because the growth of body cells depends on a certain con-

centration of a primary product of the oxidation of the cells, the archusia. This substance is soluble in blood. The cells cannot retain it in quantity. Its concentration is at all times under the control of the environment.

8. Function is likewise determined by the environment. It maintains in an environment rich in blood vessels so placed that they pass close to one end of the cell. The other end of the cell away from these vessels accumulates archusia and is active. The end near the vessels is inactive. Function is the result of an explosive breakdown of such a polarized cell. This was proved by a direct analysis of isolated functioning cells in the cultures.

9. Body cells can be made to grow not only through the accumulation of their own archusia, but by adding archusia from outside sources.

10. Function is something that maintains only in an environment that inhibits an independent growth. It occurs only in a richly vascularized tissue. Function is something imposed on the cells of the body. For them to survive in such a state they must be supplied with growth energy from other sources. This functioning tissue is formed and maintained by growth stimuli entering the blood vessels from the digestive tract. These stimuli act in the capacity of vitamins. They represent the growth energy of other living things.

11. Cancer maintains without these accessory food factors.

12. The functioning organism is evidently the result of an environment rich in growth stimulating substances supplied by preexisting lower growing and nonfunctioning forms.

13. In cancer the cell becomes independent. The cells in the aging organism must tend to revert to this state. Any tissue may be made cancerous by anything that frees the cells of the effect of their intercellular substances and an active circulation of blood.

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EXPERIMENTAL HISTOLOGICAL STUDIES

I. SOME FACTORS REGULATING THE MORPHOLOGY OF TISSUE (URETER IN VITRO)

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TEN FIGURES

Experimental biology has received in recent times a most important method in the tissue culture. In May, 1910, in Harrison's laboratory, Burrows used blood plasma for the first time as a medium for cultivating the tissues of frogs. He also cultivated for the first time the tissues of a warm-blooded animal (the tissues of chick embryos in plasma from the blood of adult chickens) and observed mitoses in cells. He thus established definitely a means of cultivating tissue.

The blood plasma has very special technical advantages over the lymph which had been used previously by Harrison in that it can be obtained in quantity and from any of a large number of animals. The proof of the great interest which this work aroused is that about 400 papers have appeared between May, 1910, and to-day. These 400 papers can be divided into three classes. The first class of papers contains a description of the method together with many modifications and a general description of the biological characteristics of the cells as they are seen in the peripheral zone of the fragments. The greatest number of the 400 publications are in this first class. In the second class, which contains a smaller

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number of these papers, the authors have made sections of these cultures and studied carefully all the morphological, histogenetic, and cytological changes which take place not only in the peripheral zone, but in the transplanted fragment. In the third class of papers the method has been used to attack bacteriological, serological, pathological, and pharmacological problems.

In spite of this very large amount of work which has been done, it is interesting that no one has come to any definite conclusions about the general characteristic reaction of cells in vitro and the relation of the behavior of these cells in the cultures to their behavior in the body. Both Chlopin ('22) and Rhoda Erdmann have noted this fact. Champy found epithelial cells after a few days to assume a spindle shape. In the same way he found muscle, bone, and cartilage cells transforming themselves into cells resembling simple fibroblasts. Champy thought these cells had dedifferentiated or, in other words, they had returned to a common primitive type. In the body he thought differentiation is the result of forces acting about these cells. The new medium releases the cells from these forces and returns them to one common type. While Uhlenhuth and Foot have accepted this theory, other authors do not believe that cells lose their specificity in the cultures (see papers by Congdon, Carrel, Lambert, Hanes, Oppel, Ebeling and Fisher). A third group of authors, Awrorow, Timofejewski, Chlopin, Maximow and Atterbury, find no dedifferentiation. They find that the cells may undergo a further differentiation in the cultures, the same as they do in the embryo.

The question arose, what is the cause of these differences of opinion and results; is it due to differences in the technic used or to other factors? After a careful study of sections of cultures prepared by Champy and others of my own in the laboratory of Tellyesniczky, I concluded that these differences in the behavior of the cells described by different authors are wholly the result of the technic which they used, and not differences in the interpretation of the same results.

To prove this more definitely, I have undertaken a study of the behavior of the cells of the ureter of young rats in various kinds of culture media.

MATERIALS AND METHODS

The ureter was chosen as a tissue very suitable for this study, because it contains epithelium, muscle, and connective tissue. These are the three tissues about which we find the greatest divergence of opinion in the literature. The ureters used in these cultures were taken from three-weeks-old white rats. At this age the rats measured 6 to 8 cm. in length. The middle centimeter of each ureter was used. This was cut into two pieces. One half was fixed and sectioned to determine its normal histology, the other half was cut into small pieces and tested in the culture media. These ureters are very small and dry quickly. In order to obviate this drying, I removed them from the animals and cut them in a dish of 0.73 per cent salt solution. The ureters were removed from the rats when they were living and under ether. Eight rats were used for these experiments, and in each case a part of both ureters was cultured. From each rat ten experimental cultures were prepared and two pieces were sectioned to determine the normal histology.

Four different media were tested in these experiments. In the first series of experiments chicken plasma was used, which I found already prepared in the laboratory in large quantities. The plasma which I used was eight months old. It had remained in small glass tubes in the ice-box during this time. These tubes were not paraffin lined. They were ordinary glass tubes which had been well cleaned with soap water, acid, and steam. In none of these tubes did one find that any clotting had taken place. The plasma, on the other hand, clotted quickly when the rat tissue was added to it. According to Burrows, paraffin is not necessary for the preservation of a fluid chicken plasma. This plasma had been prepared by him (Burrows, '19). I also found that it is as easy to prepare the chicken plasma in clean glass tubes

as in paraffin-lined ones and that the cells grow as well in the old as in fresh plasma. For all these studies I used hanging-drop cultures according to the original method of Burrows published in 1910. Burrows has found that a salt solution 0.73 per cent is physiological for rat tissue, while chicken tissue grows better in 0.9 per cent solution. For this reason, when I used chicken plasma, I diluted it with distilled water so that its osmotic pressure became equal to a salt solution of 0.73 per cent. This last per cent of salt solution he has also found to be the best for all mammalian tissues, including man.

In all experiments with plasma I diluted the plasma from one-quarter to one-half with the salt solution which is isotonic for rat tissue. This I did because Burrows ('13) had found that the zone of proliferation is increased by the addition of salt solution. The cause of this increase in the migration and growth is due in part to the action of the NaCl and in part to the dilution of the fibrin portion of the plasma. In the second series I used chicken plasma and an extract of rat embryo. The embryonic extract was made from a rat embryo 2½ cm. in length. The embryo was first cut into small pieces and ground in a porcelain mortar with salt solution. I obtained first a turbid fluid by this method. This was centrifuged and the clear supernatant part was used for the cultures. Where I used these extracts I added it one part to one part of plasma. In the third series I used rat plasma half diluted with salt solution. I found it very difficult to prepare pure rat plasma—it clots very quickly even in paraffin-lined tubes. For this reason, in the fourth series of experiments, I used plasma prepared from the blood to which a known quantity of sodium oxalate was added. In this last experiment I also added embryonic extract. To this embryonic extract I added sufficient calcium chloride to just precipitate the oxalate and reestablish the normal calcium balance of the total medium. In preparing such cultures I first placed one drop of oxalated plasma on the cover-glass; to this I then added one drop of the embryonic extract containing the calcium chloride. The two were mixed together

and the tissue added. The calcium oxalate crystals were left in the medium (Burrows, '11; Barta, '23).

In every experiment the fragment of tissue was transplanted from the first drop of medium to a second after twenty-four hours. Burrows has found that the clots will not liquefy about fragments of normal human adult connective tissue if they are transplanted after twenty to twenty-four hours from the first liquefied drop of medium to a second. After lying for this time at 37°C. in the first drop of plasma which they liquefy, they fail to liquefy the second, but grow in it. The same I found true for fragments of the ureter of rats, which also contain epithelial cells.

All cultures were studied in a warm box while living and afterward were fixed in Zenker-formol (Maximow's modification); some of the cultures were stained in toto with hematoxylin and others embedded in celloidin-paraffin and serially sectioned. These sections were stained with eosin-azure. In several instances I wanted to study the same culture stained in toto and later in sections. In such cases I stained the whole culture with hematoxylin and mounted it in cedar oil. After completing my studies of the whole culture, I then cut it loose from the cover-glass, embedded it in celloidin-paraffin, and serially sectioned it.

A STUDY OF THE CULTURES

The tissue used in all of these experiments consisted of fragments 1 mm. in length, cut through the whole wall of the ureter. Each contained epithelium, connective tissue, and muscle as they occur in the normal ureter of these rats, as shown in figure 1, the reproduction of a drawing of a longitudinal section of the ureter of a three-weeks-old rat. It is to be noticed that in these young rats the compact and refringent layer of protoplasm on the inner surface of the epithelium ('cuticula' of Hamburger) has not developed. It is also to be noticed that there are only two layers of muscle—an outer longitudinal layer and an inner circular layer. Otherwise the histology is the same as in the completely developed animals.

The reaction of the cells of the ureter in a medium of chicken plasma

In a medium of chicken plasma the connective-tissue cells migrate out from the fragment into the medium. These cells show two types of morphology. A part of them are elongated slender spindle cells set close together. Another part of them appear as irregular polygonal cells or as incomplete spindles or ovoid or round cells. Many of the smaller round

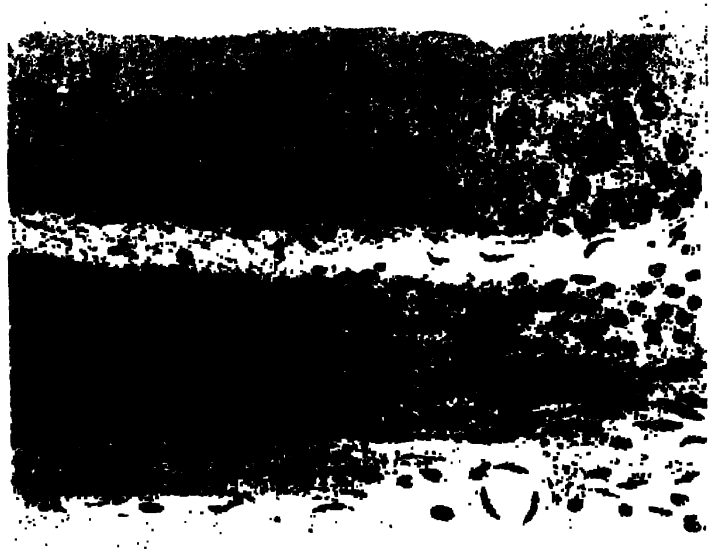

Fig. 1 Camera-lucida drawing of a longitudinal section of the ureter of a three-weeks-old rat, stained with hematoxylin, eosin, and orange G.

cells are evidently lymphocytes, while the larger ones are undoubtedly only connective-tissue cells. All of these cells contain fat-droplets and many of the more irregular polygonal cells also show phagocytosis of foreign matter. No evidence of growth or mitotic cell division was seen in any of the cells in chicken plasma. The long slender spindle cells are like the fibroblasts. A part of the polygonal cells resemble the so-called wandering phagocytic cells and another part are evidently the reticular cells of Maximow. A part

of these various types of cells are illustrated in the drawing (fig. 2).

Three rats were used for these cultures in the chicken plasma. Thirty cultures were prepared. The epithelial cells became active in only one of the thirty cultures. Serial sec-

Fig. 2 A camera-lucida drawing of the cells migrating from a fragment of ureter into chicken plasma. There are spindle-shaped fibroblasts, reticular cells of Maximow, and lymphocytes.

tions of many of these cultures showed that the epithelial cells in most cases had degenerated in situ. When the fragments are carefully cut, the epithelial cells are inside. In the one culture where they migrated out (fig. 3) the fragment had become turned inside out. In direct contact with the

chicken plasma, the epithelial cells had reacted. Away from it, they degenerate quickly, while the muscle and connective tissue remain intact for some time and show activity.

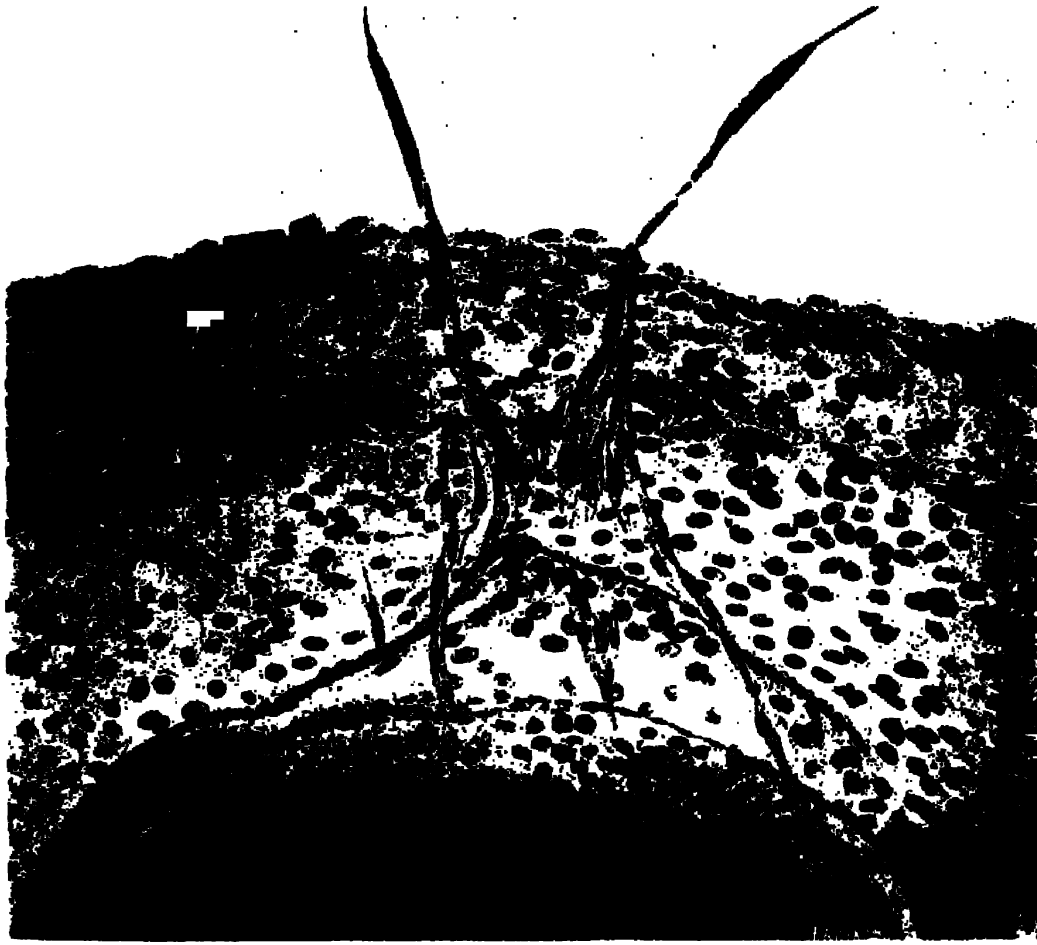

Fig. 3 A camera-lucida drawing of the one side of the fragment of the ureter in chicken plasma where the epithelial cells showed activity. A column of connective tissue is also migrating below the epithelial membrane.

In this culture it is also interesting to note that the epithelial cells did not liquefy the plasma and destroy the connective-tissue and muscle. It was possible, therefore, for the connective-tissue cells to migrate also from the fragments.. This movement of epithelial cells and their failure

to liquefy the plasma did not occur in the first drop of plasma, but in a second. The fragment had been transplanted after twenty-four hours to the second drop. The picture was made on the fourth day of the sojourn of the fragment in the second drop of plasma.

The reaction of the ureter cells in a medium composed of one part of chicken plasma and one part of an extract of a rat embryo

Twenty cultures of this kind were prepared from the ureters of two rats. In this medium the epithelium instead of the connective tissue or muscle cells migrated out from the fragment into the medium. In about one-half of these cultures an epithelial membrane surrounded the fragment completely and in the other half of the cultures membranes extended out only from one side. In this medium the connective tissue failed to react in practically every culture. The balance between connective tissue and epithelial cells in this medium is the reverse of that seen in the pure chicken plasma. The epithelial cells are dominant in this medium, while the connective-tissue cells had dominated in the culture of pure chicken plasma (fig. 4).

In this latter figure it is to be noticed that all the epithelial cells have not remained in the membrane. A few have migrated into the medium to take a spindle or spherical shape. There was no evidence, however, that these cells had otherwise changed in character. They continued to stain like epithelial cells. As noted above, connective-tissue cells may also round off in parts of the culture. They likewise continue to stain like connective-tissue cells.

The reaction of the ureter in drops of pure rat plasma diluted one-half with 0.73 per cent NaCl solution

Only a few cultures were prepared in the pure rat plasma. I had considerable difficulty in keeping the rat plasma from coagulating. In these few cases the cells did not migrate

very actively. A section made across one of the cultures of this series is shown in figure 5. It is interesting to note that the tissues in this case retain their specificity almost completely. The epithelial cells do not degenerate. They migrate, but show no mitosis. The muscle and the connective tissue also retain their characteristic form. There was no evidence of dedifferentiation in this fragment. This section is made through the middle part of the culture.

Fig. 4 A camera-lucida drawing of the ureter in a medium of chicken plasma and embryo extract. A membrane of epithelial cells has spread out from the fragment. Isolated epithelial cells at the outer border of the membrane have taken spindle and spherical shapes.

The reaction of the tissue of the ureter in oxalated rat plasma and embryonic extract

In this series twenty cultures were prepared from the ureters of two rats. In these cultures as in the cultures of pure rat plasma the connective-tissue cells failed to migrate out in any number. One also sees a very meager outward spreading of the epithelial layer. Serial sections of these cultures are interesting. Within the fragment the epithelial cells have not degenerated. They have grown most actively and divided by mitoses (fig. 6). The muscle and the con-

nective tissue, on the other hand, has undergone degeneration of a hyaline-like type. One sees in many parts of this figure no nuclear material in the muscularis and the tunica propria. Note also in the drawing of the section how the epithelial layer has thickened irregularly and that the mitoses are located in the cells nearest to the hyaline connective

Fig. 5 A camera-lucida drawing of a section of a culture of ureter in rat plasma. All tissues have retained their specificity after four days in this medium.

tissue and muscle layers. In this culture from which the section drawn in figure 6 was taken the epithelial cells had remained in the central part of the fragment. There is also a considerable amount of retroperitoneal fat attached to one side of the fragment. In one section which passes through this fat I noticed that epithelial cells have migrated from the central region and have invaded this retroperitoneal fat tissue. Here they have grown and divided (fig. 7). In

Fig. 6 A camera-lucida drawing of a section of a culture of ureter of the rat in rat plasma and embryo extract. The connective tissue and the muscle cells are degenerated. The epithelium is proliferating actively. Note the mitoses.

another section, which was cut through the periphery of this fragment (fig. 8), one sees that the epithelial cells have invaded a dense mass of connective tissue of the adventitia. Among these invading epithelial cells one finds mitosis and there are also multinuclear cells. The epithelial cells invading the connective tissue and fat have poorly defined margins. The invasion is quite typical of cancerous tissue. In other

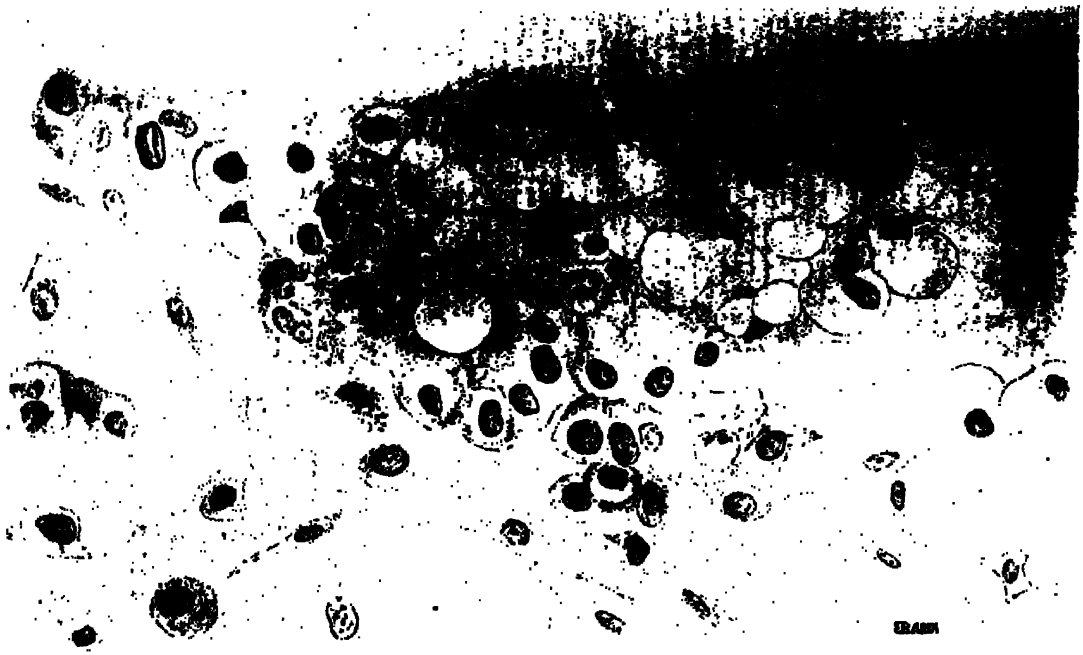

Fig. 7 A camera-lucida drawing of a section of ureter in rat plasma and embryo extract. The epithelial cells have spread from the center of the fragment of ureter and invaded the retroperitoneal fat-tissue of the fragment. Many mitotic figures are seen in these invading cells.

parts of this culture, where one finds no invasion of the fat by epithelial cells, other changes in the fat-tissue are interesting. In place of the fat-cells one finds an increased number of cells like fibroblasts and mitosis in these cells. The fat-cells have apparently lost their fat and returned to their more primitive type.

To complete the demonstration of the difference in morphology of the same tissue in different media, I have included

two microphotographs. In a medium of pure chicken plasma one sees that the connective tissue and the muscle of the ureter is most active and has proliferated. The epithelium is degenerated in this fragment (fig. 9). In a medium of rat

Fig. 8 A camera-lucida drawing of another section of a culture of ureter in rat plasma and embryo extract. In this section the epithelial cells have invaded the connective tissue of the adventitia of the fragment. There are mitosis and multinuclear cells as in cancer.

plasma containing embryonic extract (fig. 10), the epithelial cells are more active. In this section the epithelial cells have grown around the edge of the fragment and are invading the degenerated connective tissue and muscle of the ureter, *X*. At *Y* in this figure one sees one of the mitotic figures drawn in figure 6.

Fig. 9 A microphotograph of a section of the ureter in chicken plasma. It shows the connective tissue and muscle of the ureter suffering proliferation like the epithelial cells in the figure 10.

Fig. 10 A microphotograph of a section of the ureter in rat plasma and embryo extract. This section shows the proliferation of the epithelial cells. They are surrounding and invading the adventitia and one sees the degeneration of the connective tissue and muscle. Note the invasion of the adventitia by epithelial cells at *X*. A mitotic figure is shown at *Y*.

DISCUSSION

A careful survey of the picture of the various cultures studied in this series of experiments shows a variety of histological changes taking place about identical fragments of the same tissue of the same animal. Two variations which are associated with these changes in morphology are the medium and the arrangement of cells in the fragment. In a medium of chicken plasma the connective-tissue cells were alone active in fragments of the rat's ureter, excepting where the ureters were turned inside out and the epithelial cells were brought into contact with the medium. In the cultures where embryonic extracts were added to the medium there was a reverse dominance of the epithelial cells. In the cultures where the heteroplasma was used, the epithelial cells degenerated, the connective tissue remained. In the presence of the embryonic extracts the epithelial cells survived and destroyed the connective tissue and muscle. Burrows ('17) has shown that the cells in fragments of heart muscle which are in contact with the medium may live and grow at the expense of other cells within the fragments. In cultures of adult skin the epithelial cells frequently destroy the connective-tissue cells of the fragment in any medium. In cultures of embryonic skin where the mesenchyme is the more densely cellular mass the mesenchyme cells frequently destroy and live on the degenerating epithelial cells. In my experiments I have found the epithelial cell degenerating most frequently except where they are on the outside of the fragments or where the medium contains embryonic extracts. Where they are on the outside of the fragments, they migrate into the pure plasma. Where embryonic extracts are added to the medium, they prey on the connective tissue and muscle cells of the fragment.

There is no evidence in any instance that these epithelial cells revert to a connective-tissue type. They disappear simply in the presence of actively growing connective-tissue cells in certain media and under certain conditions. Under other conditions and in other media these cells either migrate

into the medium or proliferate in situ and invade the fragment. Where embryonic extracts are added to the media, these cells proliferate in situ at the expense of the connective-tissue cells. They also invade the connective-tissue areas exactly as cancer cells invade the tissue of a host. These invading cells proliferate actively and in many cases form large multinuclear cells. They have no basement membrane, but lie directly in contact with the connective-tissue cells which they are attacking.

During the growth of these epithelial cells the muscle, connective tissue, and endothelial cells are destroyed. In embryonic fragments Burrows had seen a similar active proliferation of mesenchyme cells and destruction of epithelium and blood vessels in a medium containing embryonic or tumor extracts. He has transplanted such fragments into the subcutaneous tissue of a host. When they are transplanted at the proper time they continue to grow as sarcomata and kill the animal. Fragments of these sarcomata also grow and kill other hosts as often as they are transplanted to them. There is little doubt, therefore, that we will find that these adult fragments, in which the epithelial cells are dominating and growing, will continue to grow as true cancers if they are transplanted at the proper time into a host.

The muscle and the connective-tissue cells which migrate into the medium lose all form peculiar to them in the ureter. This does not mean that they have changed their type. It simply means that these cells are simple fluid systems which become molded into shapes peculiar to their contacts. Epithelial cells coming into contact with the side of a fibrin fibril also assume a spindle shape on account of the shape of the fibril, and not because they have changed their type.

The changes which these cells suffer in the cultures are not to be called dedifferentiation in the general sense as it is used. These cells never change from one type into another. They suffer only a change in shape and in activity. We must assume that the differentiation of types or specialization is something peculiar to the embryo, but not to these types of

cultures. Whether these cells are to grow and divide or to function is, on the other hand, merely a question of adaptation to their immediate environment. Burrows has demonstrated this fact in a study of embryonic heart-muscle cells. By slight changes in the mechanical arrangement of cells he was able to transform a contracting heart-muscle cell into a growing cell which was not distinguishable from a sarcoma cell and vice versa. The contracting cell is simply a growing or migrating cell with its metabolic reactions localized at one end. It is a cell which has become polarized through specific peculiarities in the organization of its environment. Champy thought that the spindle-shaped epithelial cells which I have pictured in figure 5 had changed to a connective-tissue type. There is no evidence whatsoever that this is true. They are still epithelial cells. They take the characteristic stain. They have simply changed their shape as they are brought into contact with surfaces different from those of the body. In the same way I can find no evidence that the muscle cells do more than change their shape in the culture. The actual differentiation which determines the different layers in the embryo is not disturbed in these cultures. Again, this change in shape of the epithelial cells takes place only in certain types of media. It is not a general type of change as Champy thought it to be. The words 'dedifferentiation' and 'greater differentiation' as they have been used by different authors are misleading. I think it better to call these changes merely 'adaptation to the medium.'

In further proof for these deductions, Burrows ('13–'19) has shown that it is possible to reproduce readily the same reaction about any group of fragments of the same tissue having the same cellular content. He accomplished this only, however, when he was careful to cut the fragments the same size and to place them in the same thickness of layer of the same medium.

The tissue culture is nothing more than a biological reaction between two distinct phases: 1) the tissue fragment or cells and, 2) the medium. The comparisons become possible only

when both factors are understood. The morphological characters peculiar to no. 1 can always be modified by no. 2, the medium. In the first factor we must consider the animals from which the tissue is taken, the age of the animal (embryo or adult). In the second factor we must know the behavior of the cells to the various constituents of the medium. If we are going to criticise the various results of the different authors, we must use their technic. A proof of these deductions is the story of the cultures of the kidney in the literature. Champy found dedifferentiation when fragments of adult kidney are planted in pure plasma. Before Champy had begun his work, Burrows and Carrel had noticed tubules growing into the plasma from one out of a large number of cultures of the thyroid gland. In all other cultures the epithelial cells spread out as a membrane of cells. Atterbury found recently that when he plants pieces of nephrogenetic tissue of very young embryos on the area vasculosa he obtains a differentiation which looks much like that of the kidney. We can see from these experiments the various effects of the medium or the environment on the morphology of the cells.

By my experiments I have shown three distinct types of adaptation of the epithelial cells. The first type is shown in figure 4. In this figure one sees the epithelial cells spreading out as a membrane from the fragment and migrating out as single spindle-shaped and spherical cells. Figure 6 illustrates another form of adaptation. It is a drawing of a section of a fragment from a culture in which no epithelial membrane spread out from the fragment into the medium. The cells in this fragment have grown in situ. This growth is located chiefly in the cells nearest to the connective tissue and muscle. Corresponding with this activity in the epithelial layer, the connective tissue and muscle have undergone degeneration. A third type of adaptation of the epithelial cells to the cultures is illustrated in figures 7 and 8. In these figures one sees the migration and the growth of the epithelial cells into the connective tissue and the fatty tissue of the fragment. This type of reaction is typical of cancer.

In a few of the cultures where embryonic extract was added I have also noticed interesting changes in the fat-tissue. One sees fibroblasts forming among the previously fat-laden cells. One also sees in a few places in sections of similar cultures areas in the periphery of the fragments where the connective-tissue cells are proliferating. This proliferation is never so active, however, as that seen in the epithelium. The epithelial cells dominate in the cultures of the ureter when embryonic extracts are added to the medium. Only when the connective-tissue cells are widely separated from the epithelial cells do they become active.

From these studies it is evident that the type of reaction seen in any cell may be varied by changing the medium as well as the arrangement of cells in the fragment. It was this fact that led me to change the words and phrases 'greater differentiation,' 'dedifferentiation,' 'anaplasia to connective tissue,' 'returning to the embryonic type,' etc., to the expression 'adaptation to the media.' Using this phrase, we can eliminate the discussion in the literature and the great differences in opinion in regard to the morphological changes occurring in the tissue cultures and conclude that the different results obtained by different authors is only what is to be expected from the media and tissue they used.

Maximow has stated in a recent publication ('24) that he has seen breast epithelium grow and invade the connective tissue in a fragment of breast planted in a medium of blood plasma and an extract of bone-marrow. He could not reproduce this picture in fragments of the breast planted in a medium containing embryonic extracts. I have obtained the same result in the ureter planted in a medium of plasma and embryonic extract. Burrows has reproduced it with extracts of cancerous tissue.

In recent experiments Burrows has shown that the active principle in the embryonic extract is a product of the oxidative reaction in cells. This product acts according to its concentration to induce migration and growth in cells. It accumulates in any tissues where the cells are crowded

together and their blood supply reduced. The energy of a tissue is proportional to the concentration of this substance. Cancerous tissue like embryonic tissue grows because the cells are crowded together in an area having a reduced circulation. This substance Burrows has called the 'archusia.'

CONCLUSIONS

1. I have studied the effect of the medium on the morphology of the cells. In different media the morphology of the same tissue and cells may be different.

2. About fragments of tissue which contain epithelial cells, connective tissue and smooth muscle (ureter) planted in a heteroplasma without embryonic-tissue extract the most active cells are the connective tissue and muscle cells. The epithelium degenerates in a few days. The effect of the embryonic extract in both heteroplasma and homoplasma has been studied. In heteroplasma one sees membrane formation by the epithelial cells, but no growth and division. In homoplasma one sees an active irregular growth and mitotic figures and an invasion by the epithelial cells of the surrounding tissue.

3. Proliferation of the epithelial cells in vitro as noted in the last medium is similar to the proliferation of carcinomatous cells in the body (figs. 7, 8, and 10).

4. With a different medium and the same tissue one can reproduce differentiation, dedifferentiation, and retain the original specificity. I have called these changes therefore merely 'adaptations to the media.'

5. Many of the various changes described as peculiar for the tissue cultures have been explained by the differences in the medium used. Rhoda Erdmann states that we must have a normal type for the culture. It must be stated that every author has found evidently a normal type for the medium and the tissue which they used. The picture that they have described is the normal one for their own particular cultures. In criticising the morphological results described by these different authors, it is necessary to take into account

the media, the tissue (embryonic or adult), the thickness of the layer of media, the age of the culture, and the extract which they used.

7. Within certain limits it has been possible to modify the morphology of tissues by using the different media which have been devised. These changes are merely changes in shape and physical structure, and not fundamental changes in the chemical characteristics of the cells.

In conclusion I am indebted to the Rockefeller Foundation for the opportunity given me to work in the United States, especially Dr. Clifford W. Wells, and I wish to express my appreciation to Dr. Montrose T. Burrows for the courtesy of his laboratory, his personal interest, and the instruction he has given me.

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TISSUE CULTURES OF PLANTS*

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The continuous growth of plant tissue in sterile cultures has been recently studied by Robbins. He has found that the excised root tips from young seedlings will grow to the sixth transplant but continuous growth of meristematic tissue by frequent transplantation has not been produced. These fragments of embryonal tissue form normal differentiated root tissue with secondary and tertiary branches. Robbins used large fragments, about 10 mm. long, and cultivated them in flasks. Kotte has confirmed these results with smaller fragments, 1 mm. long, grown in test tubes. Up to the present time in cultures of plant tissue a separation and migration of the individual cells such as is found in cultures of tissue from higher animals has not been reported. In some recent experiments in which the tissue was grown in hanging drop culture for microscopic study, migration of the individual cells was noted in a root tip which had been accidentally injured. Since then, abundant migration has been produced in very small root tip fragments, less than 1 mm. long. This separation and migration of the cells from the fragment is similar to that seen in animal cells.

For these experiments squash seeds were sterilized in chloramine T and germinated in one per cent agar. When the radicle had grown to 20 or 30 mm. fragments of embryonal tissue were cut from the root tips and planted in a nutrient medium of salts, peptone, dextrose, and agar. The reaction of the medium was buffered with potassium phosphates to pH 5.6, which was the PH of a ground cotyledon extract and a ground squash seedling extract.

This migration of the individual cells, like that in the animal tissue cultures, is greatest on the surfaces of the medium, although it also occurs through the center of the drop. On the upper surface of the agar, next to the cover glass, migration has been observed to a distance of 1.7 mm. from the tissue. On the lower surface a viscid film is seen diffusing from the tissue with the cells distributed more or less evenly through it. The cells are not merely floating free in a fluid, but are held immobile against shaking. A similar surface film has been found in cultures of young embryonic animal tissue and cancer tissue, but not in cultures of older embryonic or adult tissue in a salt solution medium. The migrating cells are not the dead, plasmolyzed outer cells of the root cap, but are viable round and ovoid cells. After migration has ceased, some of the isolated cells be-

*Abstract of paper read before the Washington University Medical Society, December 10, 1923.

come plasmolyzed, others remain viable for many days.

While the migration of the plant cells resembles that of the cells of the higher animals, growth in the cultures of those small fragments is different in so far as it has been observed. The animal cells grow in mass without forming a definite structure. Mitosis has been observed in isolated cells. In the plant cultures, growth has always taken place in the fragment to form organized and differentiated root tissue. No mitosis have been seen in the cells separated from the fragment.

The data show a definite relationship between the length of the root tip excised and the growth and migration from it. Typical migration was found only in fragments under 1 mm. in length (including the root cap). The best migration was from tips between .5 and .8 mm. long. The growth was measured by increase in length of the tissue fragment. The average growth of the fragments increased directly with the size of the fragment planted. The average growth from .5 to .6 mm. fragments was .27 mm., from .6 to .7 mm. was 65 mm., and from .7 to .8 mm. was 2.27 mm.

These data suggest that plant cells and animal cells will agree in many of their fundamental physical reactions

DISCUSSION

Dr. M. T. Burrows: As Dr. Chambers has pointed out all attempts to cultivate fragments of plant tissue in vitro have lead to the growth of the total fragment rather than a separation, migration and growth of the individual cells. From fragments of the tissue of higher animals placed in the cultures the cells invariably tend to become scattered in the medium. In these tissues growth when it is seen occurs in these migrating cells and not as a growth of the mass. In cultures where plasma is used as a medium the central cells of the fragment degenerate to nourish the cells in the outer medium. Such growth does not take place in every culture but is proportional to several factors. It is greatest always about the very densely cellular fragments and maximum about fragments of these cellular tissues 1 mm. in diameter, planted in layers of medium .5 mm. in thickness. In studying the peculiarities of such a thicknesss of medium it was found that oxygen diffuses in quantities sufficient to keep red cells red no greater than .5 mm. into plasma clot and no more than 1 mm. in most tissue fragment. The exact distance for diffusion into the fragments is more difficult to measure.

In the plasma cultures it was found therefore that growth was greatest about the greatest mass of cells to be crowded into a small space where oxygen might diffuse readily. In other words there is a quantity factor in growth. Resolving that it was found that the breaking down of the cell in the

fragment and the growth without depended upon the accumulation of a product of the metabolism of the cell. Again this substance is formed in proportion to the oxygen consumed. It is apparently essential for the proper breaking down of materials and synthesis in these cells.

Dr. Chambers' experiments have shown that the same factor holds in case of the plant cells. Larger fragments grow to form roots and stems while from the smaller fragments the cells become dispersed in the medium.

These facts have given further confirmation to the idea expressed by me that the particular organization of the cancerous tissue is one of the most important factors in determining its active growth. The organization which is peculiar to cancer is a crowding of the cells and a general reduction in the blood supply. A careful study of the effect of substances and conditions which lead to cancer has shown that they lead to such a crowding of the cells. The work with cells in the cultures has shown the relation between this crowding and the growth of cells.

DR. H. LESTER WHITE: What is the source of nitrogen? Is nitrogen from peptone available for these cultures?

DR. W. H. CHAMBERS: The salt medium contains calcium nitrate, potassium nitrate, potassium phosphates, potassium chloride, magnesium sulfate and ferric sulfate. This is a modification of Pfeffer's medium which has been found adequate for the growth of normal adult plants. The nitrates are probably the main source of nitrogen. It has not been determined whether the increased growth from the addition of peptone is due to the increased supply of nitrogen.

DR. H. L. WHITE: Do the cells form fat?

DR. W. H. CHAMBERS: We have made only a few stains with osmic acid and found small stained granules, probably fat, distributed through the protoplasm of the migrated cells.

DR. LEO LOEB: Dr. Chambers concludes on the basis of his experiments that the reactions of plant tissues and animal tissues are in principle identical. However, Dr. Chambers has really compared only embryonic plant tissue with embryonic animal tissue. In these two tissues he has shown very interesting analogies to exist. But if we compare adult, fully differentiated tissues, marked differences between animal and plant tissues become noticeable. The cells of adult plant tissues in tissue cultures seem to increase in size, the thickness of the cell wall may increase, but in general cell divisions and cell movements seem to be absent. It is different in differentiated animal tissues. Thus Dr. Fleisher and I have found that fully differentiated tissues like those of kidney, thyroid may show multiplication actively by mitosis, even if no outgrowth into the

culture medium is visible. However, there is a possibility that it will be possible through special means to induce also plant tissues to divide actively. Haberlandt has found that substances extracted from leptom may call forth cell divisions in differentiated plant cells. Perhaps it will be possible through addition of appropriate substances to the culture medium to obtain a real growth of differentiated cells even in the case of plant tissues.

DR. W. H. CHAMBERS: Kotte in his article points out that the cell-division hormone of Haberlandt is formed in embryonic tissue, although he did not study the adult tissue. I think the points that Dr. Loeb has made are very well taken. When we find a medium in which we can grow adult tissue by adding hormones or other food substance, possibly then we can get the same results in adult tissues. Our experiments so far in the addition of leptom to the media have been unsuccessful.

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THE GROWTH, HYDROGEN ION CONCENTRATION, SUGAR FERMENTATION, AND SURFACE TENSION OF CULTURES OF *PSEUDOMONAS TUMEFACIENS* AND *PSEUDOMONAS CAMPESTRIS*

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THE GROWTH, HYDROGEN ION CONCENTRATION, SUGAR FERMENTATION, AND SURFACE TENSION OF CULTURES OF *PSEUDOMONAS TUMEFACIENS* AND *PSEUDOMONAS CAMPESTRIS*

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In a study of the abnormal cell growth and tumor formation in the crown gall of plants a fundamental consideration is the metabolism of the causative agent, *Pseudomonas tumefaciens*. Certain plant pathogens stimulate the host tissue to grow while others, such as the rot-producing organisms, destroy it. *Pseudomonas tumefaciens* belongs to the first class, while *Pseudomonas campestris* produces the black rot of cruciferous plants. To understand the growth-stimulating properties of *Pseudomonas tumefaciens* it became of interest to study the essential differences in those products of the metabolism of these two organisms which might be related to their pathological action in the host plant. Histological sections of tumors produced by wound inoculation with *Pseudomonas tumefaciens* show no evidence that the bacteria injure the plant cells. The only apparent reaction is growth stimulation, an excessive cell division (1). *Pseudomonas campestris* on the other hand is entirely destructive in its action. It enters the water pore of the plant, travels along the vascular system, and invades the surrounding parenchyma. The middle lamella is dissolved and the cells are eventually destroyed. Cavities are thus formed in the plant tissue (2).

The extensive research of Erwin F. Smith and his coworkers on *Pseudomonas tumefaciens* and crown gall is well known. Smith (3) cultured this organism in a medium of dextrose, peptone, and $CaCO_3$. After eight to ten weeks of growth he made a single determination of the chemical changes produced in the medium.

The qualitative analysis of the substrate at this time showed the presence of aldehyde, acetone, alcohol, acids and alkalis (ammonia). By the injection of dilute solutions of these and many other chemicals into plants, he stimulated a marked cell proliferation, so he attributed the tumor growth from *Pseudomonas tumefaciens* to the osmotic and possibly the chemical action of these substances produced intracellularly by the metabolism of the bacteria.

Harvey (4) reported a study of tumors produced on *Ricinus* by inoculation with *Pseudomonas tumefaciens*. He found that the expressed juice from the tumor tissue had a lower hydrogen ion concentration and greater catalase and oxidase activity than that of healthy stem tissue from the same plant. He deduced from this that the bacteria produced a local decrease in hydrogen ion concentration which favored the action of the respiratory enzymes. The freezing point method showed the same concentration of osmotic substances in the tumor as in the healthy tissue. However, he later noted in the wart disease of potatoes caused by *Chrysophlyctis endobiotica* an increase in hydrogen ion concentration in the tumor tissue associated with an increase in catalase activity (5).

Smith (6) has recently found by the hydrogen electrode method that the hydrogen ion concentration of older tumor tissue is usually less than that of healthy tissue while it is sometimes greater in younger tumors. He has also noted that a larger amount of acid is required by the tumor than by the healthy tissue to neutralize it to a definite endpoint.

Harding (2) has described the cultural characteristics of *Pseudomonas campestris*. He found the best growth in a neutral or an alkaline medium with inhibition by acids. Potato cylinders were changed into a solid alkaline mass giving off the odor of ammonia. The starch was almost completely converted.

For the experimental work presented in this paper Dr. Erwin F. Smith very kindly supplied a culture of *Pseudomonas tumefaciens* which he had isolated from a hop gall. During the course of the experiments the pathogenicity of this culture was confirmed in this laboratory by tumor production on *Pelargonium*

(Fig. 1). The author is indebted to Dr. L. R. Jones for the culture of *Pseudomonas campestris*, Strain 8c, transplanted stock culture of the Department of Plant Pathology, University of Wisconsin.

FIG. 1. TUMOR ON PELARGONIUM FROM INOCULATION WITH *Pseudomonas tumefaciens*

TECHNIC

The cultures were grown in 250 cc. of media in 500 cc. Florence pyrex flasks, and samples for testing were removed aseptically every 24 hours or as indicated in the data. These samples were used to determine the growth of the bacteria, the changes in the hydrogen ion concentration of the media, the quantity of reducing sugar fermented, and the changes in the surface tension of the media. Observations were made on five different media. These were a "Difco" beef peptone bouillon without dextrose and with 1 per cent dextrose, a natural potato decoction, a potato decoction with 1 per cent dextrose, and an asparagin-salt medium. The potato decoction was made according to Duggar, Severy and Schmitz (7). The asparagin-salt medium had the following formula:

Asparagin	5.0	grams.
Na_2HPO_4	5.0	"
KNO_3	2.0	"
Na_2CO_3	0.4	
$MgCO_3$	0.2	
$(NH_4)_2SO_4$	0.2	
ZnO	0.04	
$Fe_2(SO_4)_3$	0.04	
SiO_2	0.04	
Dextrose	5.0	
Distilled H_2O	1000.	cc.

The preparation of the beef bouillon, the colorimetric method of pH determination, and the general technic of the experiments have been given in an earlier publication (8).

Growth was measured by the number of viable bacteria per cubic centimeter as determined by the plate count. The Ling copper reduction method (9) was used for the quantitative sugar tests. The amount of reducing sugar is expressed as grams of dextrose per 100 cc. of media. The surface tension of the media was measured with a du Noüy tensiometer (10). The samples were thoroughly shaken and 1 cc. tested immediately to avoid the surface effects on standing reported for serum by du Noüy (11). The surface tension was measured at room temperature and corrected to 25° C. The standard for the apparatus is 77 dynes for distilled H_2O at 25° C. (10). All cultures and plates were kept at 27° C. and the plates were counted after an incubation of five days. Cultures with the same series number (see tables) were inoculated at the same time and observed together so that the unimposed conditions were identical.

DATA

The first experimental cultures were grown in plain beef bouillon without dextrose, and the growth and the hydrogen ion concentration of the media were determined daily during the period of active growth and fermentation. The results of two cultures of *Pseudomonas tumefaciens* and one of *Pseudomonas campestris* are recorded in Table 1 and charted in Fig. 2. In the culture of *Pseudomonas tumefaciens* the period of the maximum number of viable organisms was reached about the fourth day

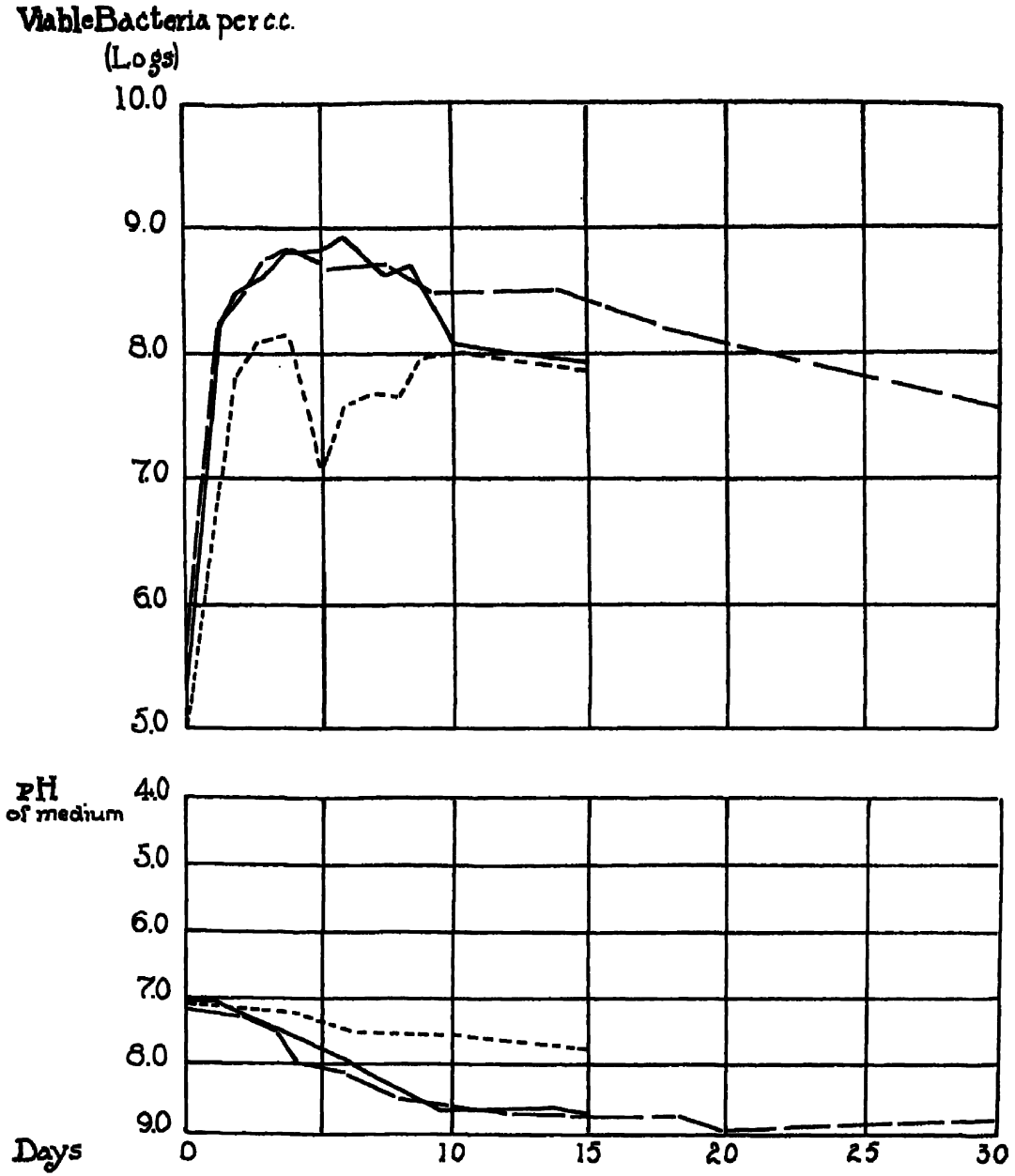

FIG. 2. GROWTH AND HYDROGEN ION CONCENTRATION OF *Pseudomonas tumefaciens* AND *Pseudomonas campestris* IN PLAIN BEEF BOUILLON AT 27° C.

——— ——— ———*Pseudomonas tumefaciens*—Table 1, Series 8.
———————*Pseudomonas tumefaciens*—Table 1, Series 7.
- - - - - - - -*Pseudomonas campestris*—Table 1, Series 7.

TABLE 1

Growth and Hydrogen Ion Concentration of Pseudomonas tumefaciens and Pseudomonas campestris in Plain Beef Bouillon at 27° C.

Days	*Bacterium tumefaciens*				*Pseudomonas campestris*	
	Series 8		Series 7		Series 7	
	Bacteria per cc.	pH	Bacteria per cc.	pH	Bacteria per cc.	pH
0	430,000	7.1	220,000	7.0	105,000	7.0
1	136,000,000	7.2	125,000,000	7.0	4,720,000	7.1
2	237,000,000	7.3	260,000,000	7.3	65,000,000	7.1
3	443,000,000	7.5	302,000,000	7.4	112,000,000	7.2
4	553,000,000	8.0	524,000,000	7.6	134,000,000	7.3
5	436,000,000	8.1	518,000,000	7.8	13,500,000	7.3
6	445,000,000	8.1	768,000,000	8.0	42,000,000	7.5
7	436,000,000	8.3	402,000,000	8.2	60,000,000	
8	373,000,000	8.5	492,000,000	8.5	56,000,000	7.5
9	331,000,000	8.5	238,000,000	8.7	95,000,000	7.5
10	361,000,000	8.5	112,000,000		105,000,000	7.5
12	359,000,000	8.6				
13		8.7		8.6		7.6
14	361,000,000	8.7				
15			95,000,000	8.7	80,000,000	7.8
18	157,000,000	8.7				
20		9.0				
35	23,000,000	8.7				

and maintained until the seventh or eighth day, after which there was a gradual decrease in number. Except for a break in the curves at five days, the growth of *Pseudomonas campestris* was similar to, although not as great as that of *Pseudomonas tumefaciens*. Alkali was produced steadily by each organism. Its production is more rapid and greater in the cultures of tumefaciens. This organism reduced the hydrogen ion concentration of the bouillon from pH 7.0 to pH 8.5 in eight days, while during the same period *Pseudomonas campestris* changed the reaction of the medium to pH 7.5.

The results of the comparative action of the two organisms in beef bouillon with one per cent of dextrose added are given in Tables 2 and 3, and Figs. 3 and 4. The growth of *Pseudomonas tumefaciens* (Fig. 3) was fairly constant in the dextrose media and considerably better than in the absence of dextrose (Fig. 2). A maximum of almost 2,000,000,000 viable bacteria per cc. were found in Series 7 and 1,000,000,000 in Series 8 and 11.

TABLE 2

Growth and Hydrogen Ion Concentration of Pseudomonas tumefaciens and Pseudomonas campestris in One Per Cent Dextrose Beef Bouillon at 27° C.

Days	*Pseudomonas tumefaciens*				*Pseudomonas campestris*			
	Series 7		Series 8		Series 7		Series 8	
	Bacteria per cc.	pH	Bacteria per cc.	pH	Bacteria per cc.	pH	Bacteria per cc.	pH
0	270,000	7.0	520,000	7.0	105,000	7.0	100	7.0
1	179,000,000	7.0	180,000,000	7.1	9,000,000	6.7	4,500	7.0
2	258,000,000	7.0	390,000,000	7.1	54,000,000	6.8	6,000,000	7.0
3	600,000,000	7.0	708,000,000	7.3	96,000,000	7.0	168,000,000	7.0
4	1,520,000,000	7.0	792,000,000	7.2	190,000,000	7.1	104,000,000	7.0
5	1,362,000,000	7.0	600,000,000	7.3	52,000,000	7.1	14,000,000	6.8
6	1,346,000,000	7.0	539,000,000	7.3	179,000,000	7.1	45,000,000	6.9
7	1,945,000,000	6.8	600,000,000	7.2	215,000,000	7.1	41,000,000	7.1
8	1,842,000,000	6.6	607,000,000	7.3	150,000,000		71,000,000	7.1
9	1,876,000,000	6.4	700,000,000	7.3	184,000,000	7.1	68,000,000	7.1
10	1,742,000,000	6.2	870,000,000	7.2	112,000,000	7.1	118,000,000	7.1
12			652,000,000	7.1			266,000,000	7.1
13		6.4		7.1		7.1		
14			955,000,000	7.1			168,000,000	7.1
15	1,110,000,000	6.5			38,000,000	7.1		
18			884,000,000	7.0			92,000,000	7.2
20				7.2				7.3
35			192,000,000	8.5				

TABLE 3

Growth, Hydrogen Ion Concentration, and Dextrose Fermentation in One Per Cent Dextrose Beef Bouillon at 27° C.

Days	*Pseudomonas tumefaciens*			*Pseudomonas campestris*		
	Series 11			Series 13		
	Bacteria per cc.	Grams Dextrose per 100 cc.	pH	Bacteria per cc.	Grams Dextrose per 100 cc.	pH
0	350,000	1.00	7.1	1,480,000	1.00	7.0
2	160,000,000	.88	7.1	157,000,000		6.8
4	175,000,000	.72	7.1	268,000,000	.91	6.9
6	418,000,000	.59	7.1	161,000,000	.53	6.9
8	736,000,000	.28	7.0	228,000,000	.44	6.9
10	900,000,000	.37	7.0	306,000,000	.06	6.7
12	680,000,000	.34	6.8		.05	6.9
14		.25	6.8	218,000,000	.05	
16	708,000,000	.12	6.8			
17				95,000,000	.03	7.4
20		.10	7.2	137,000,000	Trace	7.4
23				162,000,000	Trace	7.7
26				616,000,000	Trace	7.9
30				185,000,000	Trace	8.3
36				10,000	Trace	8.6

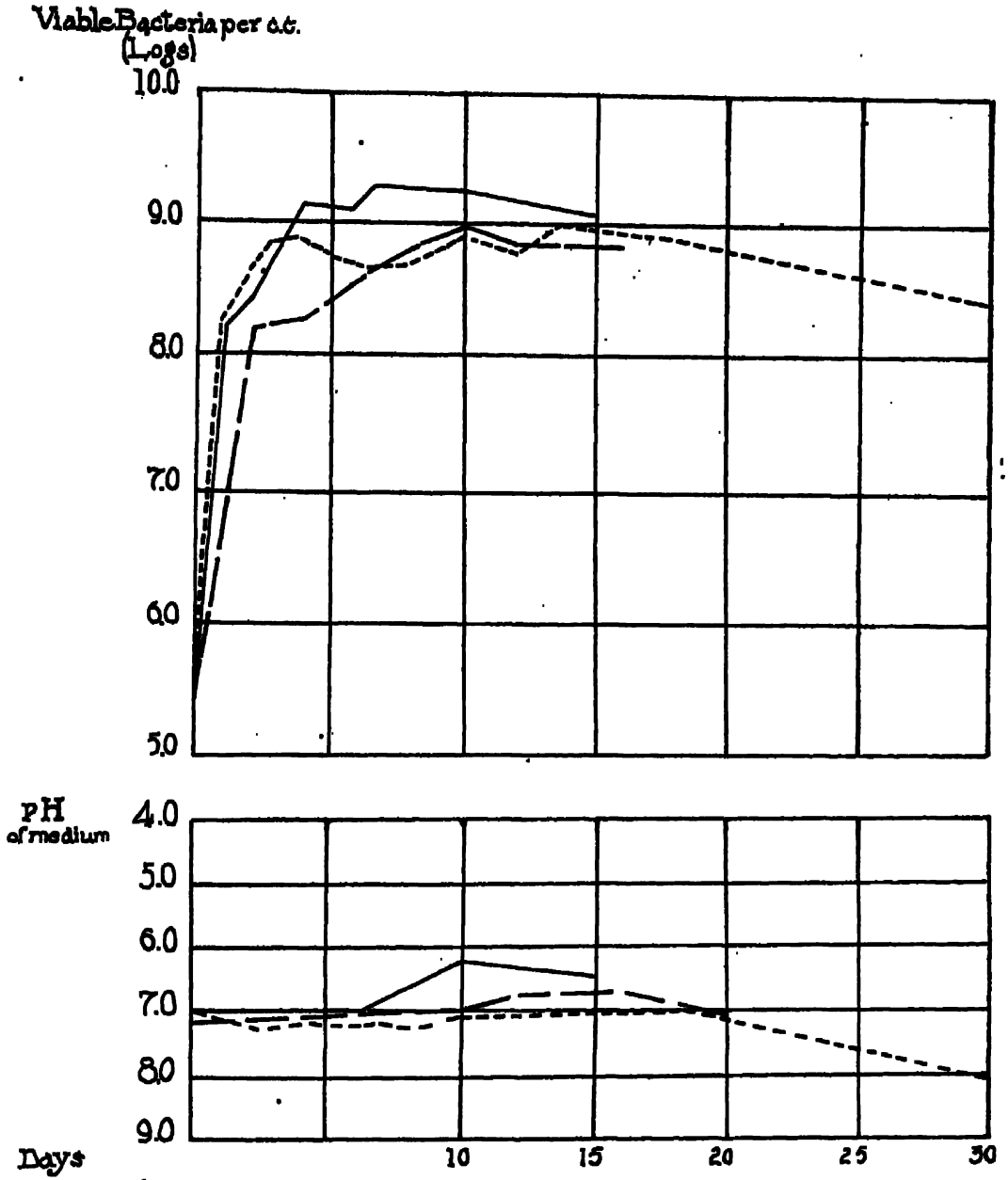

FIG. 3. GROWTH AND HYDROGEN ION CONCENTRATION OF *Pseudomonas tumefaciens* IN ONE PER CENT DEXTROSE BEEF BOUILLON AT 27° C.

–Table 2, Series 7.
–Table 2, Series 8.
· Table 3, Series 11.

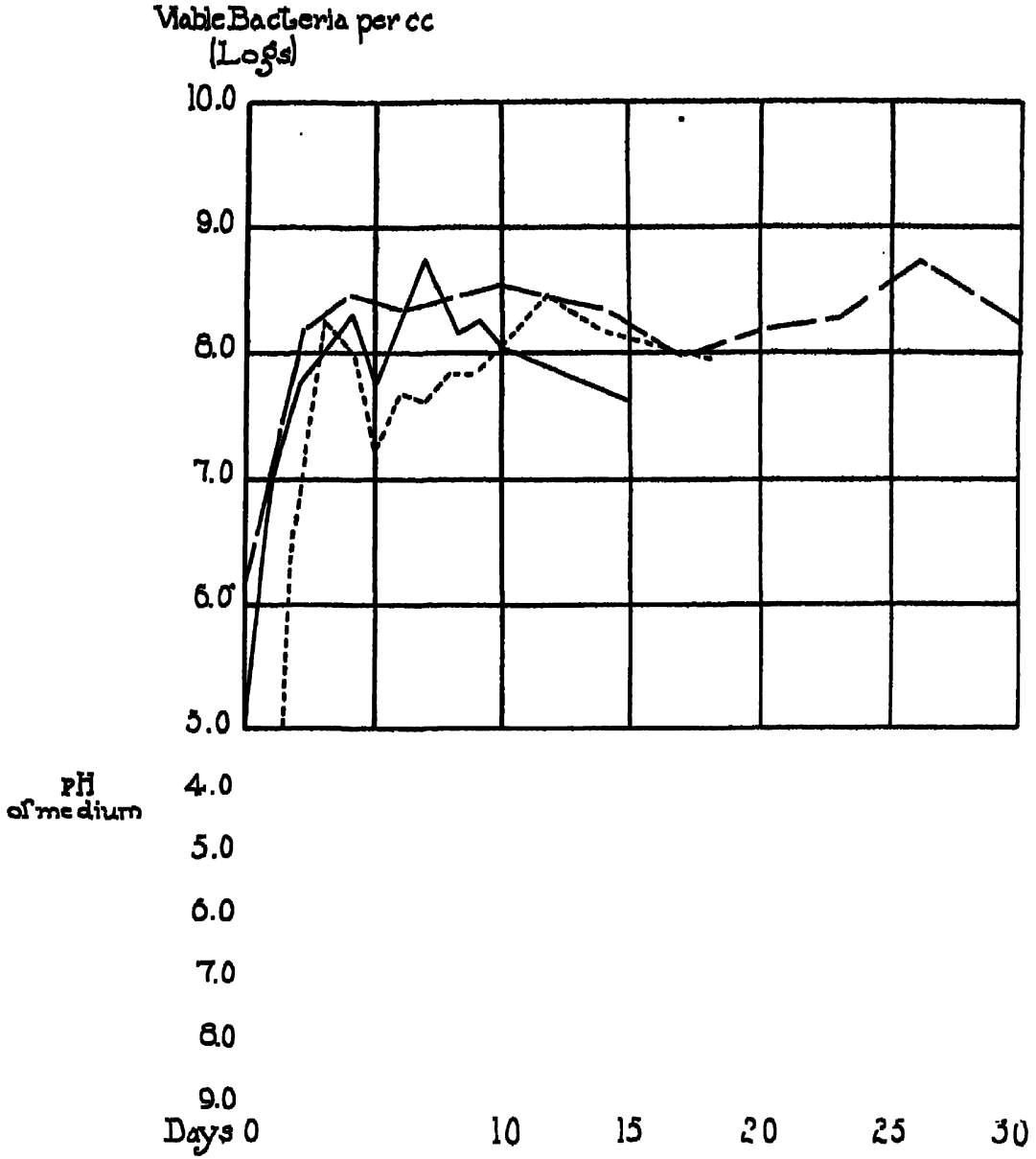

FIG. 4. GROWTH AND HYDROGEN ION CONCENTRATION OF *Pseudomonas campestris* IN ONE PER CENT DEXTROSE BEEF BOUILLON AT 27° C.

–Table 2, Series 7.
–Table 2, Series 8.
· Table 3, Series 13.

There was practically no difference between the growth of *Pseudomonas campestris* in the presence or in the absence of dextrose (Figs. 2 and 4). The hydrogen ion concentration curves of the two organisms on the other hand are very similar in this medium (Figs. 3 and 4). The reaction of the medium in each culture was maintained within a narrow zone around pH 7.0 until between the fifteenth and twentieth day, when it became gradually more alkaline. The slightly greater acid production in one of the cultures of *Pseudomonas tumefaciens* (Table 2, Series 7) might have been due to its greater growth, for in the other cultures the amount of acid produced was apparently only sufficient to neutralize the concurrent alkali production.

There was no appreciable difference between *Pseudomonas tumefaciens* and *Pseudomonas campestris* in their utilization of dextrose. Table 3 gives the determinations at intervals of two days of the amount of unfermented dextrose in the media, expressed in grams per 100 cc. of media. Fermentation proceeded in each culture with an almost constant hydrogen ion concentration until between the fifteenth and twentieth day. At this time less than .01 gram of dextrose per 100 cc. of media was left in the culture. This period corresponds to the break in the reaction curve toward the alkaline which is mentioned above. When the dextrose was diminished to a concentration of about .05 per cent, the production of alkali exceeded the acid-fermentation; consequently the hydrogen ion concentration of the cultures gradually decreased. The slight difference noted in Table 3 between the two cultures (Series 11 and Series 13) in the rate of fermentation of the dextrose is probably not indicative of a difference in metabolism between the two organisms, but most likely it is due to a difference in the two series (11 and 13),—possibly in the size of inoculum. However, the same difference is seen in a comparison of two different series of one of the organisms, *Pseudomonas tumefaciens*, in Table 5.

To observe these plant pathogens in a medium more nearly supplying their natural food, cultures were grown in a potato decoction with and without the addition of dextrose. The data are presented in Tables 4 and 5, and in Figs. 5 and 6. The

TABLE 4

Growth, Hydrogen Ion Concentration, and Fermentation in Natural Potato Decoction at 27° C.

Days	*Pseudomonas tumefaciens*			*Pseudomonas campestris*		
	Series 11			Series 13		
	Bacteria per cc.	Reduction of Copper *	pH	Bacteria per cc.	Reduction of Copper *	pH
0	66,000	0	5.6	320,000	0	5.8
2	194,000,000		6.4	47,000,000		6.0
4	928,000,000		7.1	107,000,000		6.4
6	594,000,000	0	7.7	127,000,000		6.6
8	950,000,000		8.1	116,000,000	0	6.7
10	283,000,000		8.4	137,000,000		6.9
12	200,000,000		8.5		.17	7.1
14			8.5	118,000,000	.25	
16	101,000,000		8.5			
17				686,000,000	.30	7.1
20			8.5	922,000,000	.21	7.4
23				406,000,000	.11	7.7
26				386,000,000	.12	7.7
30				255,000,000	.08	8.3
36				128,000,000	.03	7.7

* Expressed in grams of dextrose per 100 cc. of media.

TABLE 5

Growth, Hydrogen Ion Concentration, and Fermentation in One Per Cent Dextrose Potato Decoction at 27° C.

Days	*Pseudomonas tumefaciens*						*Pseudomonas campestris*		
	Series 11			Series 13			Series 13		
	Bacteria per cc.	Reduction of Copper*	pH	Bacteria per cc.	Reduction of Copper*	pH	Bacteria per cc.	Reduction of Copper*	pH
0	84,000	.98	5.6	2,680,000	1.00	5.8	400,000	1.00	5.8
2	240,000,000	.96	6.4	1,575,000,000		6.4	41,000,000		6.0
4	736,000,000	.69	6.7	2,500,000,000	.81	6.7	468,000,000	.95	6.4
6	1,050,000,000	.63	6.7	2,600,000,000	.53	6.9	178,000,000	.90	6.7
8	2,200,000,000	.42	6.7	4,200,000,000	.28	6.9	146,000,000	.86	6.7
10	3,600,000,000	.36	6.7	3,700,000,000	.10	6.9	182,000,000	.60	6.9
12	2,400,000,000	.28	6.7		.08	6.9		.59	6.9
14		.27	6.7	3,300,000,000	.07		690,000,000	.54	
16	2,250,000,000	.14	6.7						
17				1,950,000,000	.05	6.9	400,000,000	.75	7.1
20		.12	6.7	1,700,000,000	Trace	7.3	710,000,000	.91	7.5
23				1,600,000,000	Trace	7.5	792,000,000	.78	7.3
26				1,600,000,000	Trace	7.6	540,000,000	.78	7.5
30				1,700,000,000	Trace	7.5	802,000,000	.55	7.5
36				1,700,000,000	Trace	7.5	240,000,000	.60	7.4

* Expressed in grams of dextrose per 100 cc. of media.

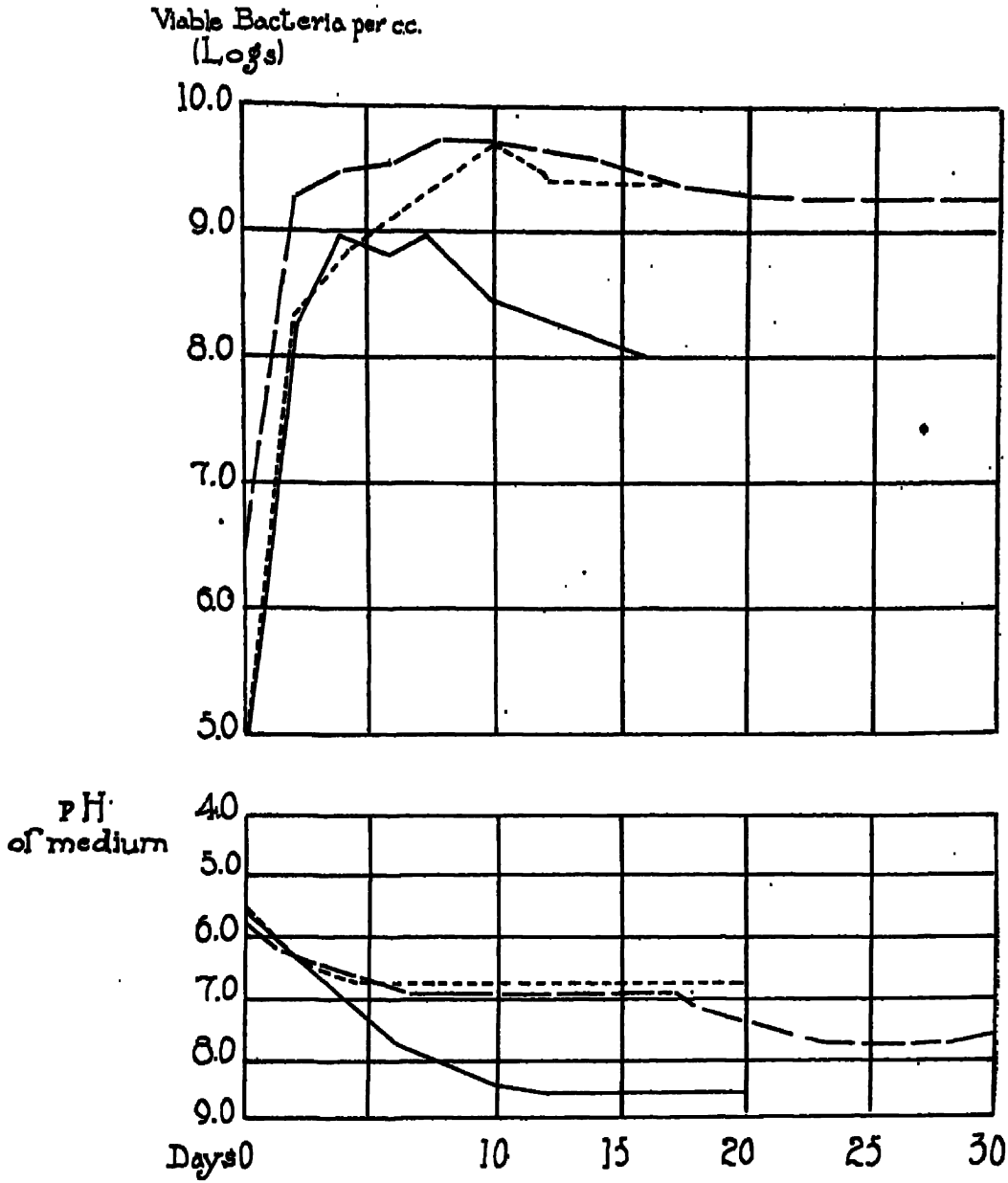

FIG. 5. GROWTH AND HYDROGEN ION CONCENTRATION OF *Pseudomonas tumefaciens* IN POTATO DECOCTION AT 27° C.

—Natural Potato Decoction, Table 4, Series 11.
-One Per Cent Dextrose Potato Decoction, Table 5, Series 11.
— One Per Cent Dextrose Potato Decoction, Table 5, Series 13.

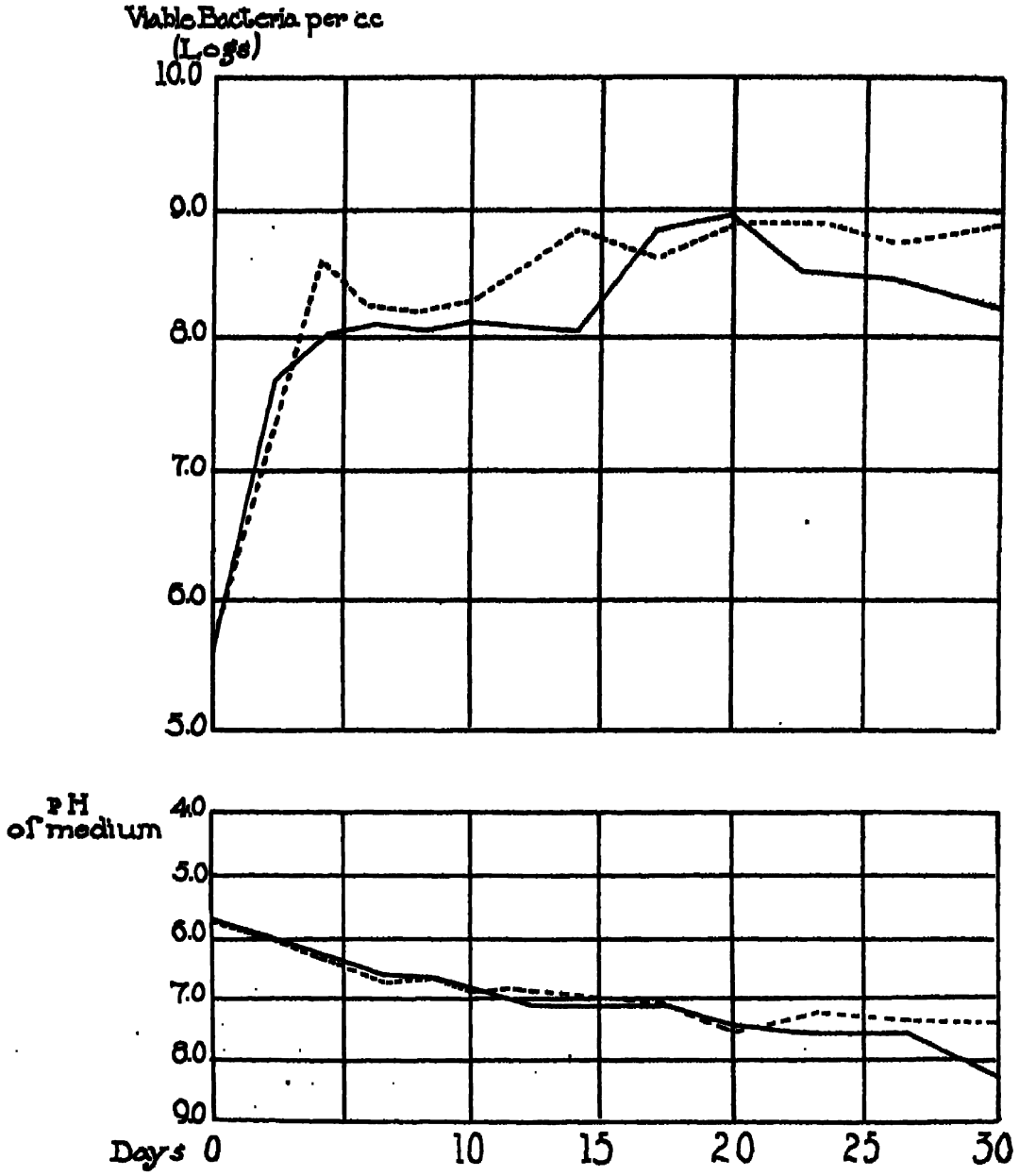

FIG. 6. GROWTH AND HYDROGEN ION CONCENTRATION OF *Pseudomonas campestris* IN POTATO DECOCTION AT 27° C.

————Natural Potato Decoction, Table 4, Series 13.

- - - - - - - -One Per Cent Dextrose Potato Decoction, Table 5, Series 13.

curves of Fig. 5 show that there was little difference in the action of *Pseudomonas tumefaciens* whether grown in beef bouillon or in potato decoction. Where dextrose was not added to the potato media (Table 4) the growth followed the general non-fermentative type, i.e. rapid growth followed by a gradual decline. A rapid production of alkali similar to that in beef bouillon is shown by the hydrogen ion concentration curve. The reaction of the medium progressed from pH 5.6 to pH 8.5 in twelve days. The dextrose potato cultures showed the best growth of all, a maximum of 4,200,000,000 viable bacteria per cc. in Series 13. The dextrose was also fermented gradually as in the dextrose beef bouillon (compare Series 11, Table 3, with Series 11, Table 5). The hydrogen ion concentration decreased the first four days from pH 5.6 to pH 6.7 in Series 11 and pH 5.8 to pH 6.9 in Series 13, and then showed no change until practically all of the dextrose had been fermented, .05 per cent or less remaining. Then the reaction continued toward the alkaline. The positive iodine test for starch showed no difference between these cultures of *Pseudomonas tumefaciens* and the uninoculated control flask of media. The dextrose determinations showed no increase in reducing sugar as in the culture of *Pseudomonas campestris*. Thus no evidence was found of a conversion of the starch or other polysaccharides by *Pseudomonas tumefaciens* in the potato decoction media.

Pseudomonas campestris exhibited no marked difference in growth or reaction in the natural and in the dextrose potato decoction cultures. The initial rise of the growth curves the first few days (Fig. 6) was followed by a second increase in numbers after the tenth day. The hydrogen ion concentration curves (Figs. 6) were practically identical up to the twentieth day. Regardless of the fermentation of the dextrose the change in the reaction grew slowly and steadily more alkaline, in contrast to the neutral reaction during the dextrose fermentation in beef bouillon (Fig. 4). The slight difference in reaction after the twentieth day may have been related to the difference in the amount of fermentable sugar present in the two cultures.

The sugar determinations on the potato decoction cultures of

Pseudomonas campestris (Tables 4 and 5) clearly illustrate the hydrolytic action of the organism on starch. In the natural potato decoction culture (Table 4) no reduction of the copper was found at the time of inoculation nor on the eighth day of growth. After that time there was an increasing reduction which reached a maximum on the seventeenth day, .30 gram per 100 cc. of media, expressed in terms of dextrose for convenience. Following this the fermentative action of the culture predominated until on the thirty-sixth day .03 per cent was found, and on the fifty-fourth day both the reduction test and iodine test for starch were negative, showing a complete conversion of the starch and fermentation of the reducing sugar.

This starch-hydrolyzing action of *Pseudomonas campestris* was confirmed in the dextrose potato decoction culture (Table 5). It occurred in both the cultures at about the same time, regardless of the fermentation of the dextrose added at inoculation. During the first fourteen days of growth (Table 5) the percentage of dextrose in the medium decreased from 1.00 to .54 per cent, then increased to .91 per cent on the twentieth day and declined again to .60 per cent on the thirty-sixth day. Dextrose and a trace of starch were still present on the fifty-fourth day. A comparison of Tables 3 and 5 shows that whereas in dextrose beef bouillon *Pseudomonas tumefaciens* and *Pseudomonas campestris* fermented dextrose with equal rapidity, in dextrose potato decoction (Series 13) the reduction test on the fourteenth day of growth showed .07 per cent present in the culture of the former and .54 per cent in that of the latter. This would indicate either a decrease in rate of fermentation or a simultaneous formation of reducing sugar by *Pseudomonas campestris* in the potato decoction.

The data for the cultures in the asparagin-salt medium are given in Fig. 7. The rapid growth of *Pseudomonas tumefaciens* during the first two days is similar to that in natural potato decoction (Table 6). The maximum of 1,108,000,000, bacteria per cc. is reached in two days. There is a subsequent inhibition to about the twelfth day which is not found in the other media. The growth of *Pseudomonas campestris* is similar to that in the dextrose beef bouillon (Fig. 4).

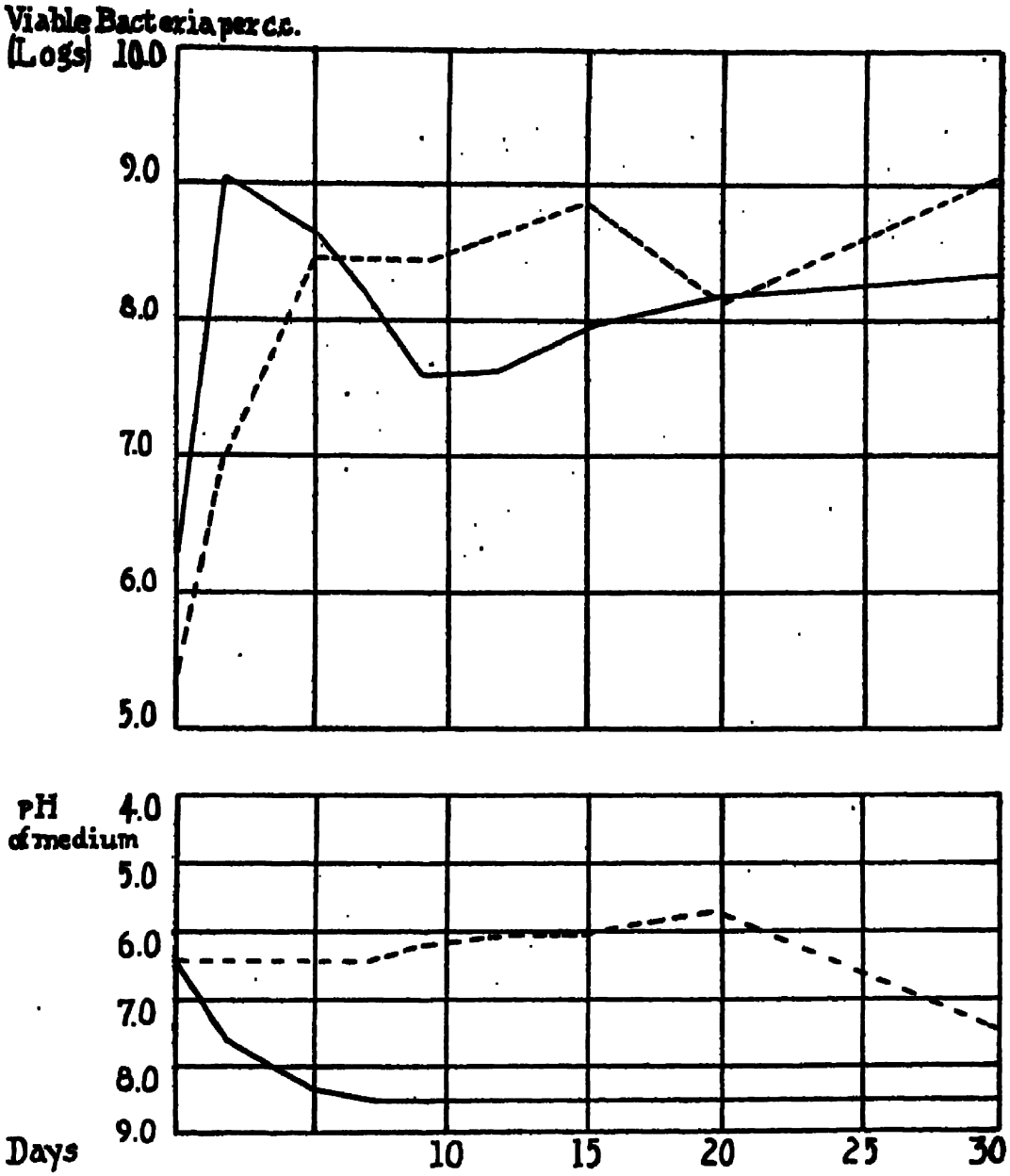

FIG. 7. GROWTH AND HYDROGEN ION CONCENTRATION OF *Pseudomonas tumefaciens* AND *Pseudomonas campestris* IN ASPARAGIN-SALT MEDIUM AT 27° C.

———————*Pseudomonas tumefaciens.*
– – – – – – – –*Pseudomonas campestris.*

TABLE 6

Growth, Hydrogen Ion Concentration, and Surface Tension of the Medium in Natural Potato Decoction and Potato Decoction + Ether Extract of Potato at 27° C.

Days	*Pseudomonas tumefaciens*						*Pseudomonas campestris*						Uninoculated Control		Temp. of Surface Tension Reading C.
	I Potato Decoction			II Potato Decoction + Ether Extract			III Potato Decoction			IV Potato Decoction + Ether Extract			V Potato Decoction + Ether Extract		
	Bacteria per cc.	pH	Dynes	Bacteria per cc.	pH	Dynes	Bacteria per cc.	pH	Dynes	Bacteria per cc.	pH	Dynes	pH	Dynes	
0	1,470,000	6.6	67.0	1,610,000	6.6	59.0	680,000	6.6	68.5	535,000	6.6	61.5	6.6	64.0	22.0
2	1,153,000,000	7.6	70.0	1,120,000,000	7.6	63.5	80,000	6.7	70.0	14,600	6.7	63.5	6.6	64.5	25.0
4	2,230,000,000	8.1	70.5	1,864,000,000	8.1	67.5	139,000,000	7.1	74.5	141,000,000	6.8	66.0	6.6	64.5	25.5
6	955,000,000	8.3	71.5	905,000,000	8.3	65.5	263,000,000	7.3	74.5	226,000,000	7.3	66.5	6.6	64.5	24.0
8	849,000,000	8.5	72.0	530,000,000	8.5	66.5	302,000,000	7.5	74.0	285,000,000	7.5	69.5	6.6	65.5	28.0
12	244,000,000	8.5	73.5	229,000,000	8.5	68.0	353,000,000	7.5	73.0	351,000,000	7.5	68.0	6.6	65.5	26.0
16	96,000,000	8.5	72.5	237,000,000	8.5	67.0	126,000,000	7.6	73.0	47,000,000	7.2	68.0	6.6	66.0	25.5
20	58,000,000	8.5	73.0	133,000,000	8.5	69.0	182,000,000	7.7	72.0	584,000,000	7.4	68.0	6.6	66.0	28.0
30	19,000,000	8.5	73.5	32,000,000	8.5	70.5	71,000,000	8.4	76.0	205,000,000	8.2	73.5	6.6	65.0	26.0

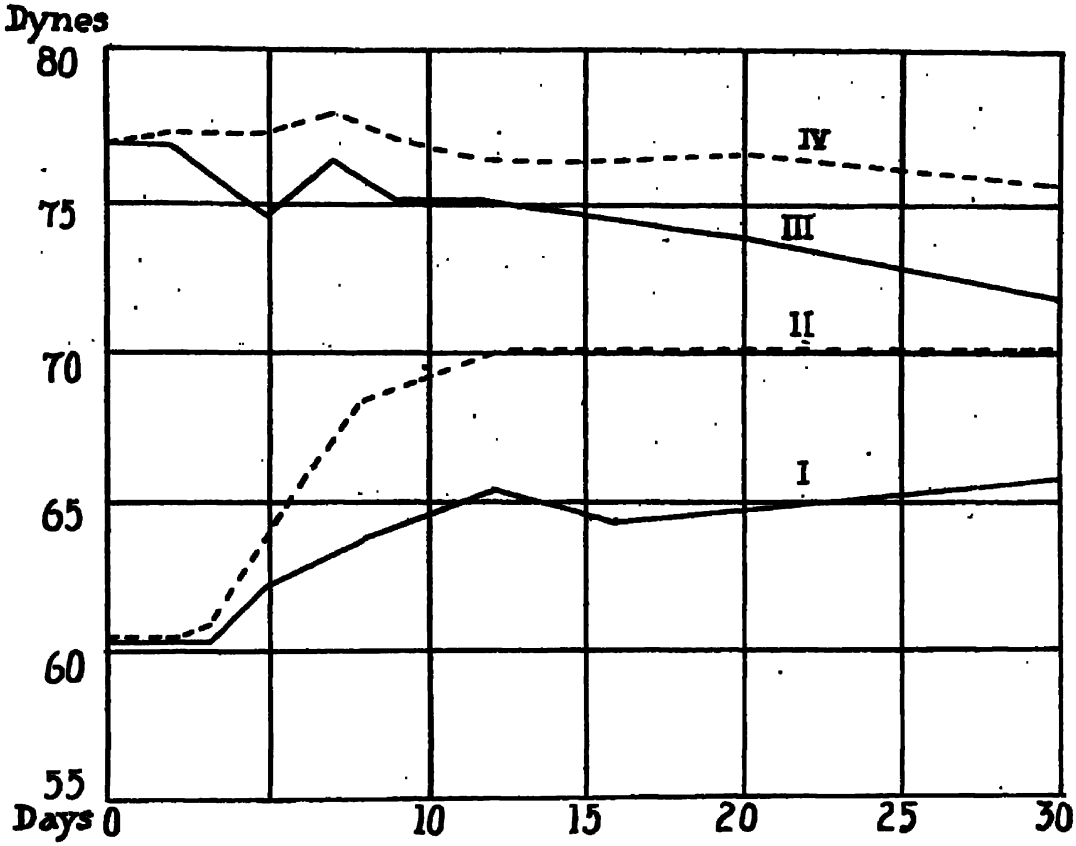

FIG. 8. SURFACE TENSION OF PLAIN BEEF BOUILLON AND ASPARAGIN-SALT MEDIUM DURING GROWTH OF *Pseudomonas tumefaciens* AND *Pseudomonas campestris* AT 27° C.

————————*Pseudomonas tumefaciens.*
– – – – – – – –*Pseudomonas campestris.*
I and II. Plain Beef Bouillon.
III and IV. Asparagin-salt medium.

A significant difference in the two organisms is found in the pH curves. Regardless of the presence of .5 per cent dextrose *Pseudomonas tumefaciens* lowered the hydrogen ion concentration of the media very rapidly, from pH 6.4 to pH 8.3 in five days. *Pseudomonas campestris* on the other hand showed an acid fermentation up to the twentieth day similar to that in dextrose beef bouillon. This was followed by an alkaline reaction.

The changes in surface tension in the medium were observed in plain beef bouillon cultures and in the cultures in the asparagin-

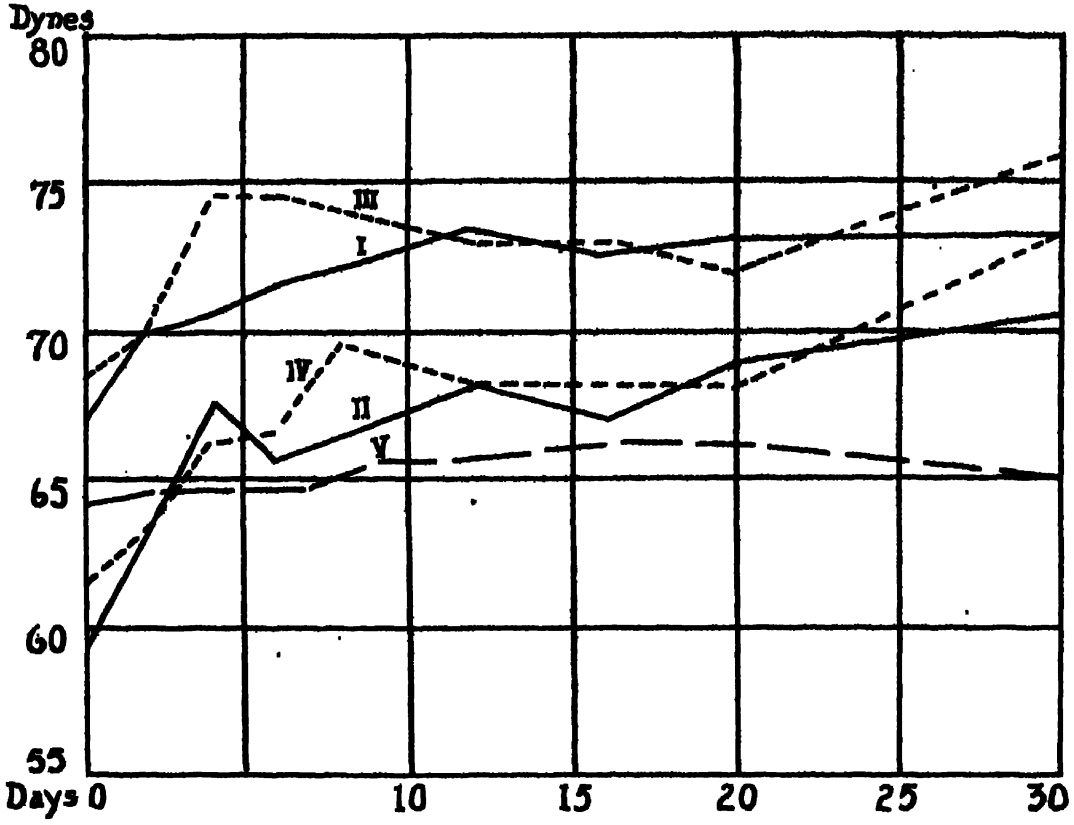

FIG. 9. SURFACE TENSION OF POTATO DECOCTION DURING GROWTH OF *Pseudomonas tumefaciens* AND *Pseudomonas campestris* AT 27° C.

I. *Pseudomonas tumefaciens* in Natural Potato Decoction.
II. *Pseudomonas tumefaciens* in Potato Decoction + Ether Extract of Potato.
III. *Pseudomonas campestris* in Natural Potato Decoction.
IV. *Pseudomonas campestris* in Potato Decoction + Ether Extract of Potato.
V. Uninoculated Control of Potato Decoction + Ether Extract of Potato.

salt medium shown in Fig. 7. These surface tension curves are presented in Fig. 8. The difference in the two organisms is quite apparent. Both organisms increase the surface tension in beef bouillon (Curves I and II). *Pseudomonas campestris*, however, increases the surface tension almost 5 dynes more than *Pseudomonas tumefaciens*. In the asparagin-salt medium (Curves III and IV) the former causes practically no change in surface tension whereas the latter decreases it 5 dynes. In the culture of *Pseudomonas tumefaciens* in beef bouillon there was no change in

surface tension (Curve 1) in the first three days of growth. During this time the number of viable bacteria increased from 420,000 per cc. to a maximum of 876,000,000 per cc. and the reaction changed from pH 6.8 to pH 7.6. The same effect is seen in the asparagin-salt medium culture (Curve III), i.e. there was no change in surface tension during the initial period of rapid growth. This is not true for *Pseudomonas campestris* (Curve 11, Fig. 7) where a maximum growth is reached in 8 days. These results indicate that *Pseudomonas tumefaciens* synthesizes a surface tension lowering substance in a simple salt medium whereas *Pseudomonas campestris* does not. The data also suggest that the surface tension changes in the media are connected with the fermentative rather than the growth reactions of the organisms.

An experiment was made to observe the ability of these organisms to destroy the natural surface tension lowering substances of the plant cells. Cultures were grown in potato decoction and in potato decoction with the addition of an ether extract of potato.[1] One of the flasks of the latter medium served as an uninoculated control of the changes in the surface tension and pH. The data are given in Table 6 and the surface tension changes are shown in Fig. 9. The cultures of *Pseudomonas tumefaciens* (Table 6, I and II) show no appreciable difference in growth or pH changes due to the presence of the ether extract. The same is true for the cultures of *Pseudomonas campestris* (Table 6, III and IV). Both of the latter cultures, however, show an unaccountable decline in growth on the second day which has not been found in the other cultures in potato decoction.

The surface tension of the uninoculated control increased progressively 2 dynes during the first twenty days and decreased 1 dyne in the next 10 days, with no change in pH. In the potato decoction cultures of both organisms (Fig. 9, Curves I and III) the surface tension changes are coincident with the initial rapid growth. They differ in this respect from the culture in beef

[1] 237 grams of potato were dried, ground in a mortar, and extracted in cold ethyl-ether. The ether filtrate was evaporated, taken up in absolute ethyl alcohol, divided equally into 3 sterile flasks, and the alcohol evaporated. The sterile potato decoction medium was then added to the flasks.

bouillon (Fig. 8, Curves I and II). Otherwise the surface tension changes produced by *Pseudomonas tumefaciens* in natural potato decoction and in beef bouillon are very similar. *Pseudomonas campestris* does not increase the surface tension in the potato decoction to the same extent as in the beef bouillon. A decrease in surface tension between the sixth and twentieth day is followed by a sharp increase.

The addition of the ether extract to the natural potato decoction in Cultures II and IV (Table 6) lowered the surface tension 8 and 7 dynes respectively. After 30 days of growth the difference between Cultures I and II and between Cultures III and IV was only 3 and 2.5 dynes respectively. This would indicate that both organisms destroy in part the surface tension lowering substances contained in the ether extract.

DISCUSSION

The growth of these two organisms is not essentially different. *Pseudomonas tumefaciens* gives the better growth in beef bouillon and in dextrose potato decoction. In the asparagin-salt medium and in the natural potato decoction the maximum growth of both organisms is approximately the same although *Pseudomonas tumefaciens* shows a more rapid early growth. The reaction of the medium for the optimum growth of *Pseudomonas tumefaciens* is about neutral. Growth inhibition appears about pH 8.0 on the alkaline side. Acid production in one culture to pH 6.2 did not seem to be inhibitory. *Pseudomonas campestris* has an optimum growth in a slightly alkaline medium, between pH 7.0 and pH 7.5. Inhibition is found at an alkalinity between pH 7.5 and pH 7.9 and at an acidity of pH 6.0.

From the pH curves alone one would conclude that *Pseudomonas tumefaciens* produced a greater increase in hydroxyl ion concentration than *Pseudomonas campestris*. A calculation of the alkali production per individual cell per hour shows that this is not the case. With the exception of the data of Table 1 the acid fermentation of the added dextrose or of the sugar formed from the hydrolysis of starch interferes with calculating the alkali production from the change in pH of the culture. Baker,

Brew, and Conn (12) have pointed out the difficulties involved in accurately computing the fermentation per cell per hour. While it is evident that the data of Table 1 are not adapted to an exact calculation, they are sufficient for a comparison between the two organisms. In as much as the growth curves follow an arithmetic rather than a geometric curve of increase during the greater part of the first four days of growth, Rahn's formula instead of Buchanan's has been used (12).

The culture of *Pseudomonas tumefaciens* (Series 7, Table 1) increased from 220,000 to 524,000,000 bacteria per cc. in four days with a change in reaction from pH 7.0 to pH 7.6, while the comparable culture of *Pseudomonas campestris* (Table 1) grew from 105,000 to 134,000,000 bacteria per cc. and changed the reaction from pH 7.0 to pH 7.3 during the same time. The titration curve of the media showed that .015 cc. of a 0.1 normal alkali solution per cc. of media were required to change the reaction from pH 7.0 to pH 7.3. The calculated alkali production per cell per hour during the first four days of growth is .000,000,000,006,6 cc. of 0.1 N alkali for *Pseudomonas tumefaciens* and .000,000,000,012 cc. for *Pseudomonas campestris*. Thus *Pseudomonas tumefaciens* produces a greater change in the pH of the culture medium in plain beef bouillon through a faster growth whereas *Pseudomonas campestris* yields almost twice as much alkali per cell per hour.

In the asparagin-salt medium the alkali production of *Pseudomonas tumefaciens* is probably greater than that of *Pseudomonas campestris*. As this medium contains .5 per cent dextrose the exact alkali production cannot be calculated. However, the latter organism only yields sufficient alkali to neutralize the acid fermentation during the first four days of growth. The culture of *Pseudomonas tumefaciens* during the same time produces an excess of alkali over acid to change the reaction of the culture from pH 6.4 to pH 8.5.

A correlation of these results on reaction changes in the media with the data of Harvey (4) and of Smith (6) on the pH of tumor tissue would indicate that *Pseudomonas tumefaciens* lowers the hydrogen ion concentration of the host plant tissue when

growing in the plant as it does that of the medium when growing in culture. The fact that *Pseudomonas campestris* produces in some cases more alkali per cell than *Pseudomonas tumefaciens* makes it appear doubtful that alkali production *per se* is a factor of importance in tumor production.

The frequent observations made in these experiments furnish a detailed picture of the action of *Pseudomonas campestris* on the plant carbohydrates. The cultures in potato decoction show the conversation of the starch and probably other polysaccharides of the plant cells to reducing sugar, the fermenting of the sugar, and coincidentally the gradual production of an alkaline reaction. *Pseudomonas tumefaciens* gave no evidence of a starch hydrolyzing action in potato decoction cultures or on starch agar plates. The fermentative activity of these organisms in culture appears to be correlated with their destructive action as parasites. *Pseudomonas campestris* shows the diastatic action in culture and in the host plant dissolves the middle lamella and eventually destroys the plant cells, whereas *Pseudomonas tumefaciens* gives no evidence of a starch-attacking property and causes a proliferation instead of a destruction of the host plant cells.

In comparing the two organisms as to the changes in surface tension which they produce in the media, the outstanding difference is the formation of a surface tension lowering substance by *Pseudomonas tumefaciens*. This is seen in the asparagin-salt medium in which *Pseudomonas tumefaciens* lowers the surface tension about 5 dynes whereas *Pseudomonas campestris* causes practically no change. The difference is confirmed in the beef bouillon cultures. Both organisms raise the surface tension but the increase caused by the former is 5 dynes less than that of the latter. In the potato decoction the difference is not so great. During the first four days *Pseudomonas tumefaciens* increased the surface tension 3.5 dynes and *Pseudomonas campestris* increased it 6 dynes. Otherwise the surface tension curves for the potato decoction cultures are much the same. It is interesting to note that both these organisms attack the surface tension lowering substances in the ether extract of the dried plant tissue.

Ayers, Rupp, and Johnson (13) and Larson (14) have shown the influence of changing the surface tension of the media on the growth of bacteria. Larson and Evans (15) have also found that with *B. coli* and *B. subtilis* the changes in surface tension during the growth vary with the different brands of peptone. The growth of the organisms is not given however. The experimental data of this paper show a similar variation in the two plant pathogens according to the medium used. A comparison of growth curves and surface tension curves of *Pseudomonas tumefaciens* in the different media indicate that surface tension changes in the cell during growth are not necessarily reflected in the media. In both the asparagin-salt media and in the beef bouillon there was no detectable change in the media during the initial period of rapid growth of the bacteria.

SUMMARY

The maximum growth of *Pseudomonas tumefaciens* and *Pseudomonas campestris* is approximately equal in the natural potato decoction and in the asparagin-salt medium. In beef bouillon and dextrose potato decoction *Pseudomonas tumefaciens* gave the better growth.

The greater alkali production by *Pseudomonas tumefaciens* than by *Pseudomonas campestris* in natural potato decoction and in plain bouillon is due to the greater growth of the former.

The alkali production per cell per hour of *Pseudomonas campestris* is about twice that of *Pseudomonas tumefaciens* in plain bouillon, but is probably not so large as that of the latter organism in the asparagin-salt medium.

Pseudomonas campestris, the destructive parasite, hydrolyzes the starch and ferments the resultant reducing sugar in potato decoction cultures. *Pseudomonas tumefaciens*, the growth stimulating organism, gives no evidence of a starch-attacking property.

A decrease in the surface tension of the asparagin-salt medium is caused by *Pseudomonas tumefaciens*. Both organisms destroy in part the surface tension lowering substances in the ether extract of dried potato tissue.

There is no change in surface tension in two of the media

during the initial period of rapid growth in the cultures of *Pseudomonas tumefaciens*.

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Fluid crystals and meristematic growth.

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(Introduced by M. T. Burrows).

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In an attack upon certain phases of the problem of meristematic growth, a cytological investigation of the cells of the squash root tip was made. Tissues fixed in fluids which preserve lipoidal structures, such as formalin-bichromate mixture, and osmic acid revealed structures which warrant description.

The growing tips were fixed by two to three weeks impregnation in 2 per cent osmic acid, after the Kopsch-Mann technique. The sections were mounted in balsam unstained. In such preparations there appeared granules of varying sizes but of uniformly high refringency. These granules were practically round, and in ordinary light the centers appear lighter than the periphery. They are present in all parts of the tip. In the growing point they are small and occur from three to six to the cell and are usually clumped in one corner or are arranged along the cell wall. Some cells, however, appear to be devoid of these characteristic granules. In the highly vacuolated cells they are much larger and fewer to the cell than in the tip. In such cases they are almost invariably found to lie at the periphery of the vacuole, close to the cell wall. The object in making these osmic acid preparations was to determine whether any structures are present which might correspond to the Golgi bodies of animal cells. It is not certain

whether these granules are actually Golgi bodies. Other granules which are not birefringent are also present and the probability is that they represent different stages in the metabolic activity of the cell. There is no evidence of a canalicular apparatus such as Bensley[1] found in the cells of the onion root tip.

When these granules were studied with the polarizing microscope they were found to be uniaxial sphaero-crystals. A very good imitation of these crystals may be obtained by making a thin smear of lecithin on a slide and treating the smear with 2 per cent osmic acid for a short time. Upon examination of such a preparation in polarized light, each of the many highly refringent droplets displays a black cross in the center, if the axis of the crystal is parallel to the optical axis of the microscope.

These birefringent droplets belong to the class of substances first termed by Lehmann, fluid crystals. Since then, Friedel[2] has suggested that this state of matter be called the mesomorphic state, being neither fluid nor crystalline, but possessing many of the properties of both states. The birefringent structures found in these cells seem furthermore to be in the state corresponding most closely to the subdivision called by him the nematic state.

The significance of the fact that many cell structures such as lipoidal granules, mitochondria, and perhaps the Golgi bodies are in the mesomorphic state in normal cell function is just beginning to be appreciated by cytologists and cell physiologists. In a valuable review of the subject, Giroud[3] has very recently called attention to the formative and proliferative power of substances in this state, with especial emphasis on mitochondria. In view of the rapid rate of division and growth of the meristematic cells, the suggestion is offered that the fluid crystalline bodies found in these cells may be important factors in this high rate of activity.

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